

599 736

COMMONWEALTH of AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

^X
We BEECHAM GROUP p.l.c.
Of Beecham House,
Great West Road,
Brentford, Middlesex TW8 9BD,
England.

hereby apply for the grant of a Standard Patent for an invention entitled:

~~"NOVEL COMPOUNDS"~~

"THIOL-CARBOXYLIC ACID DERIVATIVES"

which is described in the accompanying ~~provisional~~
complete specification.

Details of basic application(s):—

<u>Number</u>	<u>Convention Country</u>	<u>Date</u>
8630928	UNITED KINGDOM	24th December 1986
8717924	UNITED KINGDOM	29th July 1987

APPLICATION ACCEPTED AND AMENDMENTS

17-5-90

LODGED AT SUB-OFFICE

22 DEC 1987

Melbourne

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

Dated this 21st

day of December

19 87

To: THE COMMISSIONER OF PATENTS

H. d. Rimington

(a member of the firm of DAVIES &
COLLISON for and on behalf of the Applicant).

Davies & Collison, Melbourne and Canberra.



AU/1

COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952
DECLARATION IN SUPPORT OF CONVENTION
APPLICATION FOR A PATENT

In support of the Application made for a patent for an invention
entitled:

"NOVEL COMPOUNDS"

I, David Roberts, of Beecham Pharmaceuticals, Great Burgh, Yew Tree Bottom
Road, Epsom, Surrey, KT18 5XQ, England,
do solemnly and sincerely declare as follows:-

1. I am authorised by Beecham Group p.l.c. the applicant for the patent
to make this declaration on its behalf.
2. Roger Edward Markwell
Stephen Allan Smith
Ian Hughes

Of Post Office address:

Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow,
Essex CM19 5AD, England

~~A~~/All British Subject(s)

~~is~~ are the actual inventor(s) of the invention and the facts upon which
the applicant is entitled to make the application are as follows:-

by virtue of the employment of the actual inventor(s) by Beecham Group
p.l.c., the applicant would, if a patent were granted upon an application
made by the said actual inventor(s), be entitled to have the patent
assigned to it.

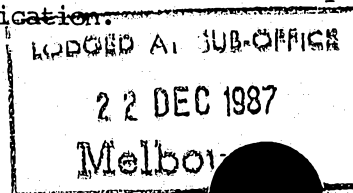
3. The basic application(s) as defined by Section 141 of the Act
~~was~~ were made in United Kingdom by Beecham Group p.l.c. as follows:


Great Britain Patent Appln. No. 8630928	filed on 24.12.86
Great Britain Patent Appln. No. 8717924	filed on 29.07.87

4. The basic application(s) referred to in the paragraph 3 of this
Declaration ~~was~~ were the first application(s) made in a Convention country
in respect of the invention the subject of the application.

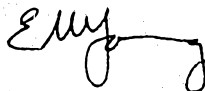
Declared at Epsom, Surrey, England

this 18th day of November, 1987




David Roberts, as Attorney for and on behalf
of the said Beecham Group p.l.c.

Witness:



(12) PATENT ABRIDGMENT (11) Document No. AU-B-82926/87
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 599736

(54) Title
THIOL-CARBOXYLIC ACID DERIVATIVES WITH COLLAGENASE- INHIBITING ACTIVITY

International Patent Classification(s)
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(22) Application Date : **22.12.87**

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(71) Applicant(s)
BEECHAM GROUP P.L.C.

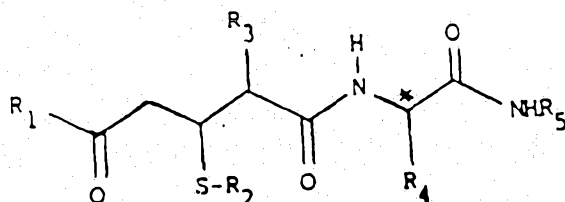
(72) Inventor(s)
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(74) Attorney or Agent
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(56) Prior Art Documents
AU 33778/84 C07C 153/11 153/09 149/273
US 4595700

(57) Claim

1. A compound of the formula (I) or a salt, solvate or hydrate thereof:



(I)

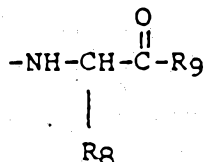
in which:

R₁ is -OH; alkoxy; aryloxy; aralkyloxy; -NR₆R₇, where each of R₆ and R₇ is hydrogen or alkyl; or R₆ and R₇, together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen,

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sulphur or optionally substituted nitrogen atom in the ring; or R_1 is a group

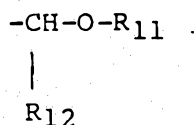


where R_8 is hydrogen; alkyl optionally substituted by -OH, alkoxy, - NR_6R_7 (wherein R_6 and R_7 are as defined above), guanidine, $-\text{CO}_2\text{H}$, CONH_2 , -SH or -S-alkyl; or $-\text{CH}_2\text{-Ar}$ where Ar is optionally substituted aryl; and R_9 is alkoxy; OH; or $-\text{NR}_6\text{R}_7$ (wherein R_6 and R_7 are as defined above);

R_2 is hydrogen; $-\overset{\text{O}}{\parallel}\text{C-alkyl}$; or $-\overset{\text{O}}{\parallel}\text{C-Z}$, where Z is optionally substituted aryl;

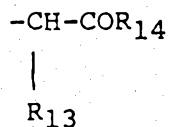
R_3 is C_{3-6} alkyl;

R_4 is hydrogen; alkyl; $-\text{CH}_2\text{-R}_{10}$ where R_{10} is optionally substituted phenyl or heteroaryl; or a group



where R_{11} is hydrogen; alkyl; or $-\text{CH}_2\text{-Ph}$ where Ph is optionally substituted phenyl; and R_{12} is hydrogen or alkyl; and

R_5 is hydrogen or alkyl; or a group



where R_{13} is hydrogen; or alkyl; and R_{14} is hydroxy; alkoxy; or $-\text{NR}_6\text{R}_7$ (wherein R_6 and R_7 are as defined above).

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10. A method of treating collagenolytic disorders, the method comprising the administration of a compound of formula (I) as defined in any one of claims 1 to 6 to a mammal in need thereof.

COMMONWEALTH OF AUSTRALIA

599 736

PATENT ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE

CLASS

INT. CLASS

Application Number:

Lodged:

Complete Specification Lodged:

Accepted:

Published:

Priority:

Related Art:

This document contains the
amendments made under
Section 39.

and is correct for printing:

NAME OF APPLICANT: BEECHAM GROUP p.l.c.

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Great West Road,
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NAME(S) OF INVENTOR(S) Roger Edward MARKWELL
Stephen Allan SMITH
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ADDRESS FOR SERVICE: DAVIES & COLLISON, Patent Attorneys
1 Little Collins Street, Melbourne, 3000.

COMPLETE SPECIFICATION FOR THE INVENTION ENTITLED:

~~"NOVEL COMPOUNDS"~~

"THIOL-CARBOXYLIC ACID DERIVATIVES"

The following statement is a full description of this invention,
including the best method of performing it known to us :-



NOVEL COMPOUNDS

"THIOL-CARBOXYLIC ACID DERIVATIVES"

The present invention relates to novel thiol-carboxylic acid derivatives, processes for their preparation and their use in medicine. In particular, the present invention relates to their use as collagenase inhibitors for treating arthritic and other diseases.

The range of therapeutic applications of the collagenase inhibitors described hereinafter reflects the fundamental role of collagen within the connective tissue matrix throughout the body, and extends to many diseases not primarily due to collagen destruction but involving tissue remodelling, as these will also be susceptible to clinical intervention with collagenase inhibitors. In particular, inhibition of collagenases released from synovial and skin fibroblasts, chondrocytes, peripheral mononuclear cells, keratinocytes and gingival tissue, as well as inhibition of collagenase stored in polymorphonuclear leucocytes (PMNLs) should be of therapeutic value, and the present compounds are envisaged as having application against these and related mammalian collagenases.

Specifically, collagenase inhibitors will provide useful treatments for arthritic diseases such as rheumatoid arthritis and osteoarthritis, soft tissue rheumatism, polychondritis and tendonitis; for bone resorption diseases such as osteoporosis, Paget's disease, hyperparathyroidism and cholesteatoma; for the recessive classes of dystrophic epidermolysis bullosa; for periodontal disease and related consequences of gingival collagenase production or of PMNL collagenase production following cellular infiltration to inflamed



gingiva; for corneal ulceration e.g. that induced by alkali or other burns, by radiation, by vitamin E deficiency or retinoid deficiency; and for systemic chemotherapy of cancer, where collagenase has been implicated in the neovascularization required to support tumour survival and growth, and in the penetration of tumour cells through the basement membrane of the vascular walls during metastasis. A collagenase inhibitor may also be of use in some post-operative conditions such as colonic anastomosis in which collagenase levels are raised.

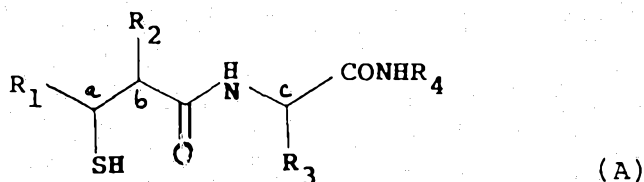
As a particular example of the therapeutic value of collagenase inhibitors, chronic arthritic diseases lead to extensive loss of the collagen and proteoglycan components within the cartilage and bone of the affected joints. Neutral metalloproteases, especially collagenases and proteoglycanases, are currently thought to be the major enzymes involved.

These enzymes have been detected in extracts of synovial and cartilage tissue, and have also been extensively studied in tissue cultures of these organs. Apart from control of the biosynthesis or secretion of the enzymes, the most significant natural regulation of the activity of collagenase and proteoglycanase in the normal and diseased state, is considered to be the production of inhibitors such as the Tissue Inhibitor of Metalloproteases (TIMP) and α_2 -macroglobulin. An imbalance between the levels of proteolytic enzymes and natural inhibitors will allow destruction of the connective tissue components to proceed.

Restoration of the enzyme-inhibitor balance by treatment with synthetic inhibitors of collagenase thus

offers a useful therapy for a wide range of connective tissue diseases in which collagenolytic activity is a causative or major contributory factor.

US Patent No. 4,595,700 discloses compounds of the formula (A):



in which:

R₁ represents lower alkyl, phenyl or phenyl lower alkyl;

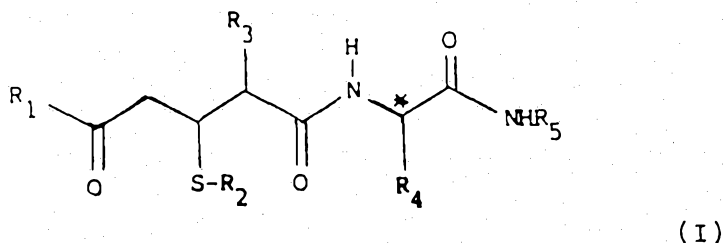
R₂ and R₄ represent lower alkyl; and

R₃ represents lower alkyl, benzyloxyalkyl, alkoxybenzyl or benzyloxybenzyl wherein the oxyalkyl or alkoxy moiety contains 1 to 6 carbon atoms and a, b and c represent chiral centres with optional R or S stereochemistry.

These compounds are described as inhibitors of collagenase, useful in the treatment of rheumatoid arthritis and related diseases in which collagenolytic activity is a contributing factor.

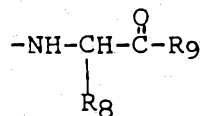
A novel class of thiol-carboxylic acid derivatives has now been discovered, which are collagenase inhibitors and thus of potential utility in the treatment of diseases in which collagenolytic activity and tissue remodelling is implicated.

According to the present invention there is provided a compound of general formula (I), or a salt, solvate or hydrate thereof:



in which:

R₁ is -OH; alkoxy; aryloxy; aralkyloxy; -NR₆R₇, where each of R₆ and R₇ is hydrogen or alkyl, or R₆ and R₇ together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen, sulphur or optionally substituted nitrogen atom in the ring; or ^{R₁ is} a group



where R₈ is hydrogen; alkyl optionally substituted by -OH, alkoxy, -NR₆R₇, guanidine, -CO₂H, CONH₂, SH, or S-alkyl; or -CH₂-Ar where Ar is optionally substituted aryl; and R₉ is alkoxy; OH; or -NR₆R₇;

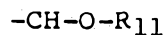
R₂ is hydrogen; or acyl, such as $\overset{\text{O}}{\parallel}\text{-C-alkyl}$ or $\overset{\text{O}}{\parallel}\text{-C-Z}$,



where Z is optionally substituted aryl;

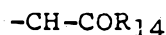
R₃ is C₃₋₆ alkyl;

R₄ is hydrogen; alkyl; -CH₂-R₁₀ where R₁₀ is optionally substituted phenyl or heteroaryl; or a group



where R₁₁ is hydrogen; alkyl; or -CH₂-Ph where Ph is optionally substituted phenyl; and R₁₂ is hydrogen or alkyl; and

R₅ is hydrogen; alkyl; or a group



where R₁₃ is hydrogen; or alkyl; and R₁₄ is hydroxy; alkoxy; or -NR₆R₇.

Unless otherwise specified, each alkyl or alkoxy group is a C₁₋₈ group, more preferably C₁₋₆, and may be a straight chain or branched.

Optional substituents for aryl, phenyl and heteroaryl groups may be selected from OH, C₁₋₆ alkyl, C₁₋₆ alkoxy and halogen.

Examples of R₁ are hydroxy; C₁₋₆ alkoxy, such as methoxy, ethoxy or t-butyloxy; benzyloxy; and -NR₆R₇ in which R₆ is hydrogen, and R₇ is hydrogen or C₁₋₈ alkyl such as methyl or ethyl; or -NR₆R₇ is N'-methyl-N-

piperazinyl or N-morpholinyl. Other examples of R_1 are

$-\text{NH}-\text{CH}_2-\text{COOH}$, $-\text{NH}-\text{CH}_2-\text{CONH}_2$, $-\text{NH}-\text{CH}_2-\text{CO}_2\text{Et}$,
 $-\text{NH}-\text{CH}_2-\text{CO}_2^t\text{Bu}$, $-\text{NH}-\underset{\text{iBu}}{\text{CH}}-\text{CO}_2\text{H}$, and $-\text{NH}-\underset{\text{iBu}}{\text{CH}}-\text{CO}_2^t\text{Bu}$.

R_1 is preferably alkoxy, such as C_1 -4 alkoxy, especially methoxy; amino; alkylamino, especially methylamino or $-\text{NH}-\text{CH}_2-\text{CO}_2\text{H}$.

When R_2 is $-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{Z}$, Z is preferably an optionally substituted phenyl group.

Examples of R_2 are hydrogen; $-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{CH}_3$ and benzoyl.

R_3 is preferably a C_4 alkyl group, such as n-butyl, iso-butyl or sec-butyl, especially iso-butyl.

When R_4 is $-\text{CH}_2-\text{R}_{10}$ and R_{10} is heteroaryl, values for R_{10} include 5- or 6- membered monocyclic and 9- or 10- membered bicyclic heteroaryl of which 9- or 10- membered bicyclic heteroaryl is preferred.

In addition, 5- or 6- membered monocyclic and 9- or 10- membered bicyclic heteroaryl preferably contain one or two heteroatoms selected from nitrogen, oxygen and sulphur which in the case of there being more than one heteroatom may be the same or different. When R_{10} is 9- or 10- membered bicyclic heteroaryl the two rings are preferably fused with one 5- or 6- membered ring containing a single heteroatom.

R_4 is preferably iso-butyl; benzyl; or C_1 -6 alkoxybenzyl, such as 4-methoxybenzyl;



1-(benzyloxy)ethyl or 9- or 10- membered fused bicyclic heteroarylmethyl such as 3-indolylmethyl.

Examples of R₅ include hydrogen; alkyl, such as methyl or ethyl, preferably methyl; and 1-(methoxycarbonyl)-ethyl.

The compounds of formula (I) may form salts with bases e.g. sodium hydroxide. When a basic nitrogen atom is present, the compounds of formula (I) may form acid addition salts e.g. with hydrochloric acid. Such compounds form part of the present invention.

Where compounds of formula (I), or salts thereof, form solvates such as hydrates, these also form an aspect of the invention.

The compounds of formula (I) have at least one asymmetric centre and therefore exist in more than one stereoisomeric form. The invention extends to all such forms and to mixtures thereof, including racemates, and diastereoisomeric mixtures.

Preferred isomers are those having the S configuration at the chiral centre marked with an asterisk in formula (I).

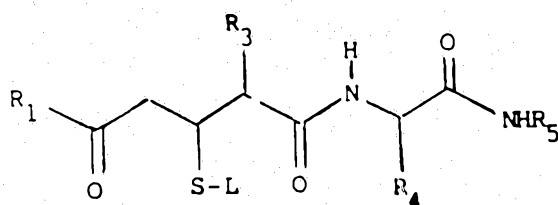
The compounds of formula I or their salts, solvates or hydrates are preferably in pharmaceutically acceptable or substantially pure form. By pharmaceutically acceptable form is meant, inter alia, of a pharmaceutically acceptable level of purity excluding normal pharmaceutical additives such as diluents and carriers, and including no material considered toxic at normal dosage levels.

A substantially pure form will generally contain at least 50% by weight, preferably 75%, more preferably 90% and still more preferably 95% or 99% or more of the compound of formula I or its salt or solvate.

One preferred pharmaceutically acceptable form is the crystalline form.

The present invention provides the compounds of formula (I) or pharmaceutically acceptable salts or solvates thereof for use as active therapeutic agents, particularly as agents for treatment of musculo-skeletal disorders resulting from collagenolytic activity, particularly arthritic diseases, and tissue remodelling, and also for the systemic chemotherapy of cancer.

The present invention also provides a process for the preparation of a compound of formula (I) in which R₂ is hydrogen, which comprises cleaving a group L from a compound of formula (II):



(II)

wherein L is L₁ which is a conventional sulphur protecting group or L₂ which is a group R-S- where R is any organic residue such that the group R-S- provides a cleavable disulphide bond, and R₁, R₃, R₄ and R₅ are as defined for formula (I).

Typically a protecting group L_1 is a substituted benzyl group, such as alkoxybenzyl e.g. 4-methoxybenzyl or an aliphatic or aryl acyl group such as acetyl or benzoyl. When L_1 is acyl it is of course identical to R_2 , so these compounds of formula (II) are themselves compounds of the invention. When L is R-S- then typically the compound of formula (II) is a dimer of the compound of formula (I) in which R_2 is hydrogen.

When L is L_1 , and L_1 is a substituted benzyl sulphur protecting group, such as 4-methoxy benzyl, then L_1 may be removed by treatment with mercury acetate in trifluoroacetic acid containing anisole followed by reaction with hydrogen sulphide in dimethyl formamide, in a procedure analogous to that described in Chem. Pharm. Bull 1978, 26, 1576.

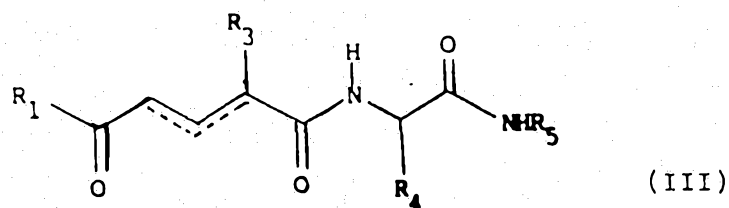
When L_1 is an acyl group it may be removed by treatment with a base, for example aqueous ammonia or dilute aqueous sodium hydroxide, or by treatment with an acid, for example methanolic hydrochloric acid.

When L is L_2 the dimerized compound may be split at the disulphide link by treatment with zinc and hydrochloric acid or by passing hydrogen sulphide through the solution.

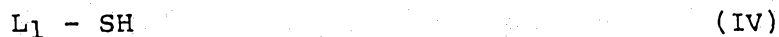
Other conventional methods for removing sulphur protecting groups or cleaving disulphide bridges may also be used.

Compounds of formula (II) in which L is L_1 may be prepared by treating a compound of formula (III):



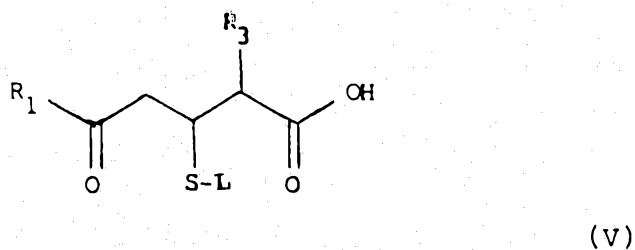


in which R_1 , R_3 , R_4 and R_5 are as defined in formula (I) with a thiol of formula (IV):

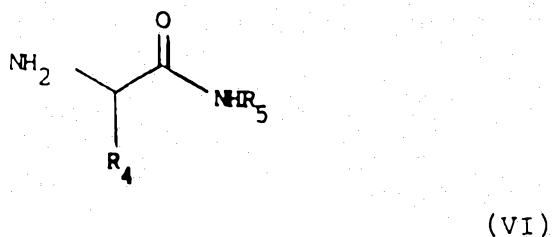


in which L_1 is as defined in formula (II). When L_1 is R_2 , the compounds of formula (II) thereby produced are compounds of the invention.

Compounds of formula (II) may also be prepared by treating a compound of formula (V):



in which L , R_1 and R_3 are as defined in formula (II), with a compound of formula (VI):



in which R_4 and R_5 are as defined in formula (I).

The reaction is preferably carried out in the presence of a coupling agent, such as N,N-dicyclohexylcarbodiimide.

Compounds of formula (II) in which L is L_2 may also be prepared by oxidative coupling, with iodine or oxygen, of a compound of formula (I) in which R_2 is hydrogen.

Compounds of formula (II) can be converted to further compounds of formula (II) while retaining the same group L, which group in turn can be cleaved to form compounds of the invention in which R_2 is hydrogen.

For example, those compounds of formula (II) in which R_1 is -OH may be prepared under acid conditions by hydrolysis of compounds in which R_1 is alkoxy, aryloxy or aralkyloxy or by hydrogenolysis of compounds in which R_1 is benzyloxy or substituted benzyloxy in the presence of a catalyst such as palladium black.

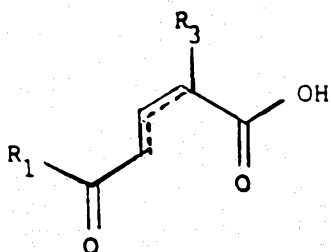
Those compounds of formula (II) in which R_1 is - NR_6R_7 may be prepared from compounds in which R_1 is -OH by treating the latter compounds with an amine of formula NHR_6R_7 in the presence of a coupling agent such as N,N-dicyclohexylcarbodiimide or N-ethyl-N'-dimethylaminopropylcarbodiimide.

Compounds of formula (II) in which R_1 is - $NH-CH(R_8)-COR_9$ may be similarly prepared from compounds in which R_1 is OH by treatment with amine derivatives of formula $NH_2CH(R_8)COR_9$ where R_9 is an alkoxy or amine group, followed by hydrolysis to give an R_9 hydroxy group, if desired.

In addition, compounds of the invention in which R_2 is acyl can be converted to further compounds of the invention with concomitant cleavage of the acyl group to give compounds of formula (I) in which R_2 is hydrogen.

For example, those compounds of formula (I) in which R_1 is OH and R_2 is hydrogen may be prepared by hydrolysis of compounds in which R_1 is alkoxy, aryloxy or aralkyloxy and R_2 is acyl under basic conditions such as treatment with dilute sodium hydroxide.

The intermediate compounds of formula (III) may be prepared by treating a compound of formula (VII):



(VII)

in which R_1 and R_3 are as defined in formula (I), with a compound of formula (VI) as defined above.

The thiols of formula (IV) are known compounds.

The intermediate compounds of formula (V) may be prepared by treating a compound of formula (VII), as defined above, with a thiol of formula (IV).

It may be necessary or convenient to protect the carboxyl function in compounds of formula (VII), for example by esterification, prior to treatment with the thiol of formula (IV) and subsequently remove the protecting group under acid conditions.

The compounds of formula (VI) are either known amino acid derivatives or can be made from these derivatives by known methods.

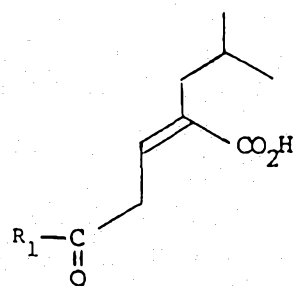
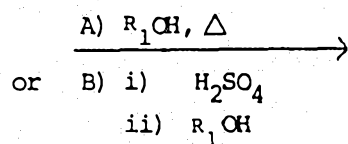
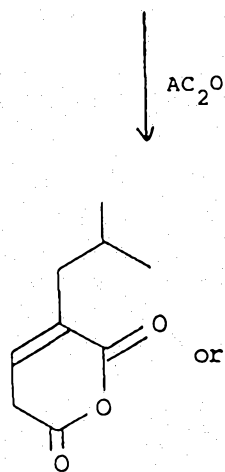
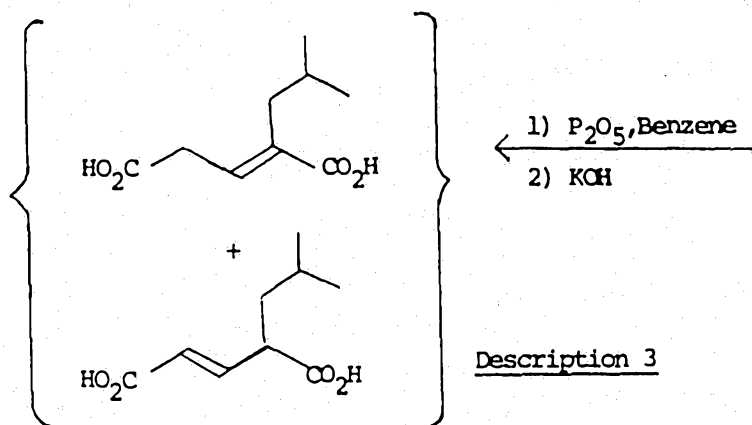
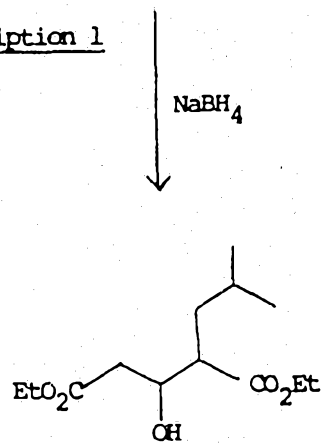
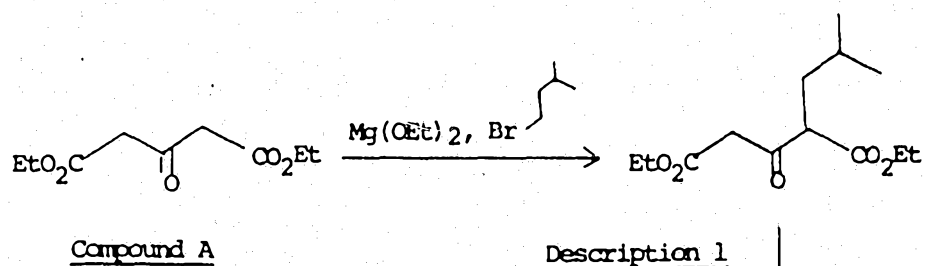
The intermediates of formulae (III), (V) and (VII) disclosed herein are in some forms novel compounds and form an aspect of the present invention as do the described processes for their preparation.

The preparation of certain compounds of formula (VII) is illustrated in the following reaction Scheme I, using compounds in which R₃ is iso-butyl.

The starting material, compound A in Scheme I is a known compound, diethyl 3-oxopentanedioate.

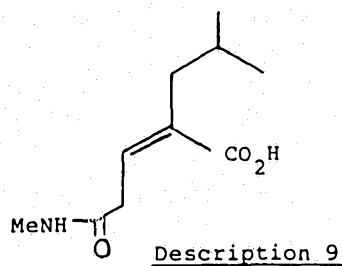
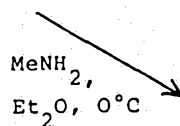
The preparation of compounds of formula (V) from compounds of formula (VII) in which the carboxyl group is protected as described above is illustrated in reaction Scheme 2, using compounds in which R₁ is methylamino, R₃ is iso butyl, L is benzoyl and the carboxyl group is protected as a t-butyl ester.

SCHEME 1

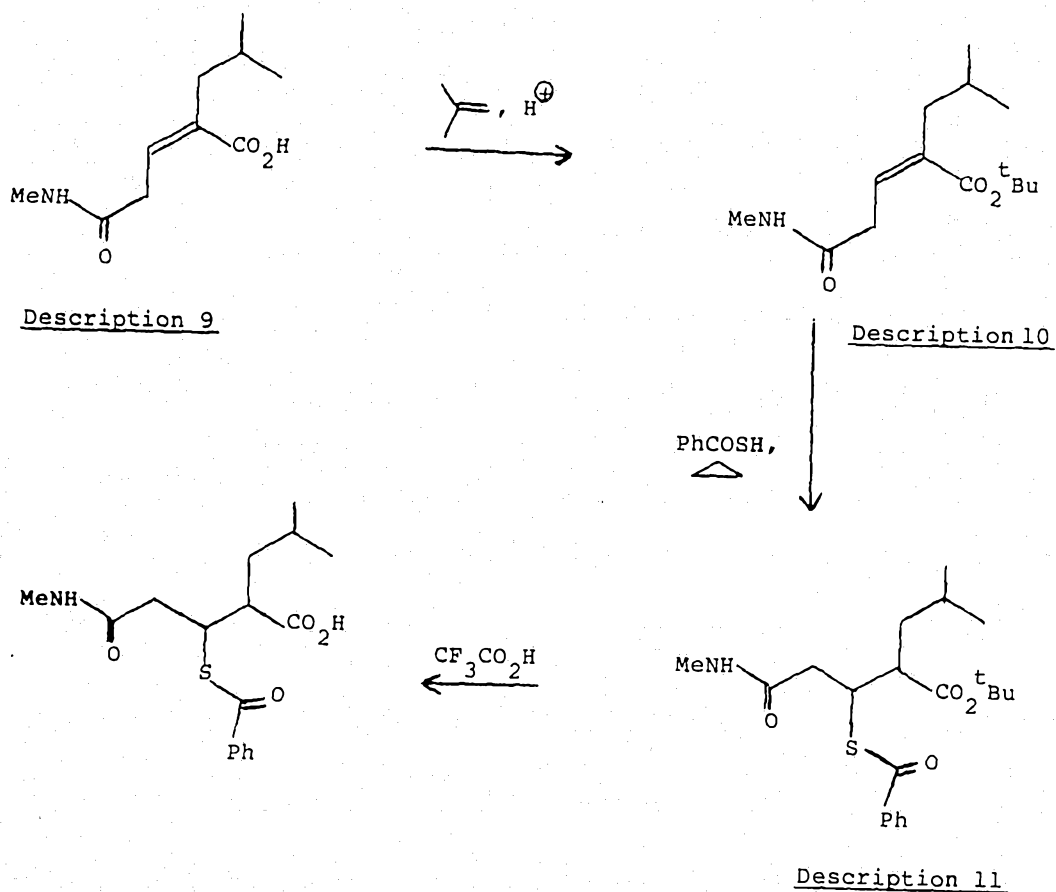


Description 5: $\text{R}_1 = \text{MeO}-$

Description 7: $\text{R}_1 = \text{PhCH}_2\text{O}-$



SCHEME 2



Where obtainable, pharmaceutically acceptable salts of the compounds of formula (I) may be formed conventionally by reaction with the appropriate acid or base. Solvates may be formed by crystallization from the appropriate solvent.

As mentioned previously, the compounds of formula (I) exist in more than one diastereoisomeric form. Where the processes of the invention produce mixtures thereof, the individual isomers may be separated one from another by chromatography, e.g. HPLC.

Alternatively, separate diastereoisomeric compounds of formula (I) can be obtained by using stereoisomerically pure starting materials or by separating desired isomers of intermediates at any stage in the overall synthetic process, and converting these intermediates to compounds of formula (I).

The present invention further provides a pharmaceutical composition, which comprises a compound of formula (I), or a pharmaceutically acceptable salt, solvate or hydrate thereof, and a pharmaceutically acceptable carrier.

A composition of this invention is useful in the treatment of rheumatism and arthritis and in the treatment of other collagenolytic conditions.

A composition of the invention, which may be prepared by admixture, may contain a diluent, binder, filler, disintegrant, flavouring agent, colouring agent, lubricant or preservative in conventional manner. These conventional excipients may be employed in conventional manner, for example as in the preparation of compositions of related peptide enzyme inhibitors, such

as the ACE inhibitor captopril.

A composition of the invention may be adapted for oral, topical, percutaneous, rectal or parenteral - intravenous, intramuscular, sub-cutaneous, intradermal or intra-articular administration, but oral administration is preferred.

Preferably, a pharmaceutical composition of the invention is in unit dosage form and in a form adapted for use in the medical or veterinarial fields. For example, such preparations may be in a pack form accompanied by written or printed instructions for use as an agent in the treatment or prophylaxis of any of the disorders mentioned above.

The suitable dosage range for the compounds of the invention may vary from compound to compound and may depend on the condition to be treated. It will also depend, inter alia, upon the relation of potency to absorbability and the mode of administration chosen.

The compound or composition of the invention may be formulated for administration by any route, the preferred route depending upon the disorder for which treatment is required, and is preferably in unit dosage form or in a form that a human patient may administer to himself in a single dosage.

Compositions may, for example, be in the form of tablets, capsules, sachets, vials, powders, granules, lozenges, reconstitutable powders, or liquid preparations, for example solutions or suspensions, or suppositories.

The compositions, for example those suitable for oral administration, may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycollate or microcrystalline cellulose; or pharmaceutically acceptable setting agents such as sodium lauryl sulphate.

Solid compositions may be obtained by conventional methods of blending, filling, tabletting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. When the composition is in the form of a tablet, powder, or lozenge, any carrier suitable for formulating solid pharmaceutical compositions may be used, examples being magnesium stearate, starch, glucose, lactose, sucrose, rice flour and chalk. Tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating. The composition may also be in the form of an ingestible capsule, for example of gelatin containing the compound, if desired with a carrier or other excipients. For example, in a hard gelatin capsule containing the required amount of a compound of the invention in the form of a powder or granulate in intimate mixture with a lubricant, such as magnesium stearate, a filler, such as microcrystalline cellulose, and a disintegrant, such as sodium starch glycollate.

01 Compositions for oral administration as liquids may be
02 in the form of, for example, emulsions, syrups, or
03 elixirs, or may be presented as a dry product for
04 reconstitution with water or other suitable vehicle
05 before use. Such liquid compositions may contain
06 conventional additives such as suspending agents, for
07 example sorbitol, syrup, methyl cellulose, gelatin,
08 hydroxyethylcellulose, carboxymethylcellulose,
09 aluminium stearate gel, hydrogenated edible fats;
10 emulsifying agents, for example lecithin, sorbitan
11 monooleate, or acacia; aqueous or non-aqueous vehicles,
12 which include edible oils, for example almond oil,
13 fractionated coconut oil, oily esters, for example
14 esters of glycerine, or propylene glycol, or ethyl
15 alcohol, glycerine, water or normal saline;
16 preservatives, for example methyl or propyl
17 p-hydroxybenzoate or sorbic acid; and if desired
18 conventional flavouring or colouring agents.
19
20

21 The compounds of this invention may also be
22 administered by a non-oral route. In accordance with
23 routine pharmaceutical procedure, the compositions may
24 be formulated, for example for rectal administration as
25 a suppository or for parenteral administration in an
26 injectable form. For injection, the compounds of the
27 invention may be presented in an aqueous or non-aqueous
28 solution, suspension or emulsion in a pharmaceutically
29 acceptable liquid, e.g. sterile pyrogen-free water or a
30 parenterally acceptable oil or a mixture of liquids,
31 which may contain bacteriostatic agents, anti-oxidants
32 or other preservatives, buffers or solutes to render
33 the solution isotonic with the blood, thickening
34 agents, suspending agents or other pharmaceutically
35 acceptable additives. Such forms will be presented in
36 sterile unit dose form such as ampoules or disposable
37 injection devices or in multi-dose forms such as a

bottle from which the appropriate dose may be withdrawn or a solid form or concentrate which can be used to prepare an injectable formulation.

For topical and percutaneous administration, the preparations may also be presented as an ointment, cream, lotion, gel, spray, aerosol, wash or skin paint or patch.

A unit dose for inflammatory diseases will generally contain from 10 to 1000 mg and preferably will contain from 10 to 500 mg, in particular 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 mg. The composition may be administered once or more times a day, for example 2, 3 or 4 times daily, so that the total daily dose for a 70 kg adult will normally be in the range 10 to 3000 mg. Alternatively, in particular for injection, the unit dose will contain from 2 to 200 mg of a compound of the invention and be administered in multiples, if desired, to give the desired daily dose.

The present invention additionally provides a method of treating a collagenolytic condition such as rheumatism and/or arthritic conditions, or cancer, or other diseases in which enzyme-mediated breakdown of connective tissue components plays a role in mammals, such as humans, which comprises administering an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt, solvate or hydrate thereof, to the mammal.

The present invention also provides the use of a compound of formula (I) or a pharmaceutically acceptable salt, solvate or hydrate thereof, for the manufacture of a medicament for use as an active therapeutic substance, particularly in the treatment of

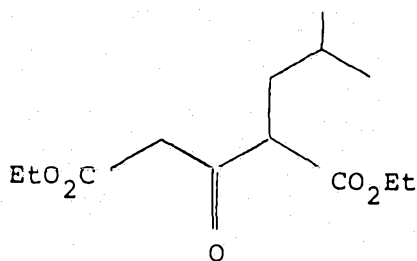
- 21 -

collagenolytic conditions, such as rheumatism, cancer,
bone disorders, skin diseases, periodontal disease or
corneal ulceration, in mammals.

The following Descriptions and Examples illustrate the
preparation of compounds of the invention and the
subsequent biological data illustrates their
pharmacological activity. All temperatures are
expressed in °C.

Description 1

Diethyl 2-(2-methylpropyl)-3-oxopentanedioate (D1)

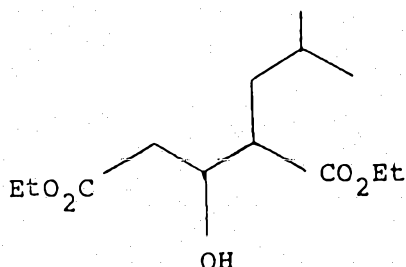


(D1)

Diethyl 3-oxopentanedioate (45.5ml, 0.25mol) was added to a suspension of magnesium ethoxide (prepared from magnesium turnings (9g, 0.375mol) and iodine (0.1g) in dry ethanol (275ml)), and the mixture was heated under reflux for 90 min. 1-Bromo-2-methylpropane (54ml, 0.5mol) was added, and the reaction was heated under reflux for 16h. Further 1-bromo-2-methylpropane (27ml, 0.25mol) was added to the boiling mixture, and after 3h the solvent was evaporated in vacuo. The residue was partitioned between 2N hydrochloric acid and ether. After extracting the aqueous phase with ether, the combined organics were washed successively with water, 5% sodium bicarbonate, water and brine, then were dried (Na₂SO₄) and evaporated in vacuo. The residue was distilled to give the title compound as a colourless oil (40g, 62%), bp 118-120°C (1mmHg).
 δ (CDCl₃): 0.9 (6H, d, J=6Hz), 1.25 (6H, t, J=7Hz), 1.4-1.9 (3H, m), 3.5 (2H, s), 3.7 (1H, d, J=9Hz) and 4.2 (4H, q, J=7Hz).

Description 2

Diethyl 3-hydroxy-2-(2-methylpropyl)pentanedioate (D2)

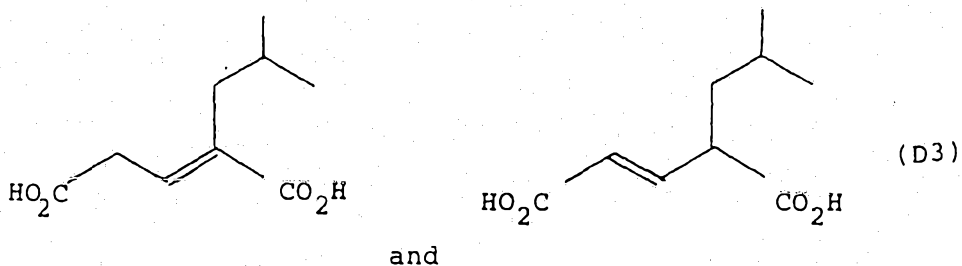


Sodium borohydride (1.1g, 29mmol) was added to an ice-cold solution of diethyl 2-(2-methylpropyl)-3-oxopentanedioate (7.5g, 29mmol) in ethanol (75ml). The mixture was stirred at 5-10°C for 2h, then 2N hydrochloric acid (18ml) was added slowly at 0°C, and the mixture was stirred at room temperature for 45 min. The mixture was diluted with water (400ml), then was extracted with ethyl acetate (4 x 100ml). The extracts were washed with water (2 x 100ml) and brine (100ml), then were dried (Na₂SO₄) and evaporated in vacuo to leave the title compound as an oil (7.5g, 100%).

δ (CDCl₃): 0.9 (6H, d, J=5Hz), 1.15 (6H, t, J=7Hz), 1.1-1.8 (3H, m), 2.2-2.7 (3H, m), 3.3 (1H, brs), 3.7-4.1 (1H, m) and 4.1 (4H, q, J=7Hz).

Description 3

2- and 4-(2-Methylpropyl)pent-2-enedioic acids (D3)



Diethyl 3-hydroxy-2-(2-methylpropyl)pentanedioate (26g, 100mmol) was added to a stirred suspension of phosphorus pentoxide (21.3g, 150mmol) in dry benzene (220ml), and the mixture was heated under reflux for 3h. Water (200ml) was added to the cooled mixture to break down the brown gum. The layers were separated and the aqueous phase was extracted with ether (4 x 150ml). The combined organics were washed with water (150ml) and brine (300ml), then were dried (Na₂SO₄) and evaporated in vacuo to leave a brown oil.

The above oil was dissolved in a solution of potassium hydroxide (16.8g, 300mmol) in 80% ethanol (300ml), and the mixture was heated under reflux for 3h. The ethanol was evaporated in vacuo and the residue was diluted with water (400ml), and washed with ether (150ml). The aqueous solution was acidified with 5N hydrochloric acid, then was extracted with ether (4 x 150ml). The combined extracts were washed with brine (2 x 100ml), then were dried (Na₂SO₄) and evaporated in vacuo to leave a mixture of the two title compounds as an oil which slowly solidified (14.3g, 77%). The mixture of acids was used in the following stage without separation.

However, it was possible to separate the isomers by crystallisation from ether/pentane, which gave 2-(2-methylpropyl)pent-2-enedioic acid as a solid, m.p. 129-135°C (chloroform).

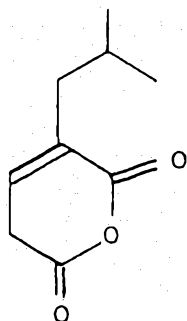
δ (DMSO d_6): 0.9 (6H, d, $J=6\text{Hz}$), 1.3-2.0 (1H, m), 2.15 (2H, d, $J=7\text{Hz}$), 3.2 (2H, d, $J=7\text{Hz}$), and 6.8 (1H, t, $J=7\text{Hz}$).

Evaporation of the mother liquors gave 4-(2-methylpropyl)pent-2-enedioic acid as an oil.

δ (CDCl₃): 0.9 (6H, d, $J=6\text{Hz}$), 1.4-2.3 (3H, m), 3.4 (1H, m), 5.85 (1H, d, $J=15\text{Hz}$) and 7.0 (1H, dd, $J=15, 7\text{Hz}$).

Description 4

2-(2-Methylpropyl)pent-2-enedicarboxylic anhydride (D4)

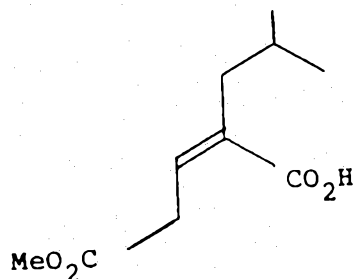


(D4)

A mixture of 2- and 4-(2-methylpropyl)pent-2-enedioic acids (14.3g, 77mmol) in acetic anhydride (40ml) was heated under reflux for 2½h. The acetic anhydride was evaporated in vacuo to leave a brown oil, which was distilled to give the title compound as a low-melting yellow solid (5.8g, 45%), b.p. 145-155°C (5mmHg).
 δ (CDCl₃): 0.9 (6H, d, J=6Hz), 1.5-2.4 (3H, m), 3.5 (2H, brd, J=4Hz), 6.5 (1H, t, J=4Hz).

Description 5

4-Methoxycarbonyl-2-(2-methylpropyl)but-2-enoic acid
(D5)



(D5)

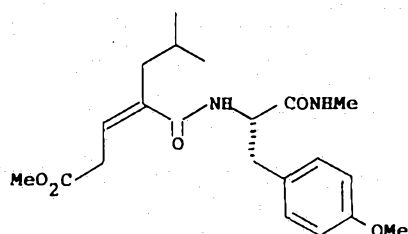
2-(2-Methylpropyl)pent-2-enedicarboxylic anhydride
(960mg, 5.7mmol) was dissolved in concentrated
sulphuric acid (10ml), then immediately poured into
ice-cooled methanol (50ml). The solution was diluted
with water (100ml) and was extracted with ether (3 x
50ml). The extracts were washed with brine, then dried
(MgSO₄) and the solvent was evaporated in vacuo to
leave the title compound as a pale yellow solid (960mg,
84%), mp 40-45°C (hexane).

δ (CDCl₃): 0.9 (6H, d, J=6Hz), 1.4-2.0 (1H, m), 2.2
(2H, d, J=6Hz), 3.55 (2H, d, J=7Hz), 6.2 (1H, t, J=7Hz)
and 9.6 (1H, brs).

ν_{max} (nujol): 1735, 1690 and 1635cm⁻¹

Description 6

6-Methyl-4-[[[1-[(methylamino)carbonyl]-2-(4-methoxy-phenyl)ethyl]amino]carbonyl]hept-3-enoic acid, methyl ester (D6)



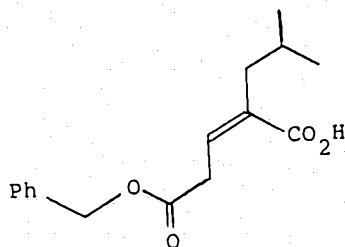
(D6)

N,N-Dicyclohexylcarbodiimide (515mg, 2.5mmol) was added to an ice-cooled solution of 4-methoxycarbonyl-2-(2-methylpropyl)but-2-enoic acid (500mg, 2.5mmol) and O-methyl-L-tyrosine N-methylamide (520mg, 2.5mmol) in dry dichloromethane (30ml), and the mixture was stirred at room temperature for 18h. The precipitated solid was filtered off and washed with a little dichloromethane, then the organic solution was washed successively with 1N hydrochloric acid (20ml), 1N sodium bicarbonate (20ml), and water. The solution was dried (MgSO₄) and evaporated in vacuo. Column chromatography (30g silica) of the residual solid, eluting with 5% methanol/dichloromethane gave the title compound (520mg, 53%), m.p. 142-144°C (dichloromethane/pentane).

δ (CDCl₃): 0.82 (3H, d, J=7Hz), 0.83 (3H, d, J=7Hz), 1.58 (1H, m), 2.18 (2H, d, J=7Hz), 2.72 (3H, d, J=5Hz), 3.02 (2H, m), 3.16 (2H, d, J=7Hz), 3.70 (3H, s), 3.78 (3H, s), 4.57 (1H, m), 5.70 (1H, brs), 6.32 (1H, t, J=7Hz), 6.45 (1H, d, J=8Hz), 6.83 (2H, d, J=8Hz) and 7.12 (2H, d, J=8Hz).

Description 7

4-Benzyloxycarbonyl-2-(2-methylpropyl)but-2-enoic acid
(D7)



(D7)

The title compound was prepared in 91% yield from benzyl alcohol by the method of D5.

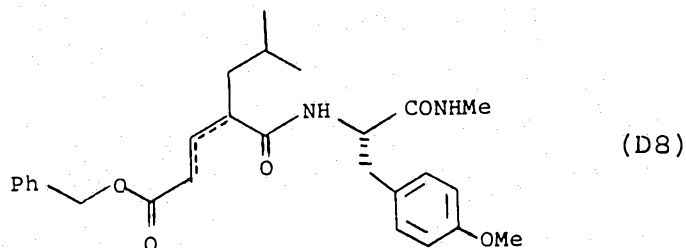
A sample recrystallized from ether/pentane gave white needles, m.p. 72-73.5. (Found: C, 69.55; H, 7.38.

C₁₆H₂₀O₄ requires C, 69.54; H, 7.30%).

δ (CDCl₃): 0.9 (6H, d, J=6Hz), 1.4-2.0 (1H, m), 2.16 (2H, d, J=6Hz), 3.63 (2H, d, J=7Hz), 5.08 (2H, s), 6.23 (1H, t, J=7Hz) and 7.2 (5H, s).

Description 8

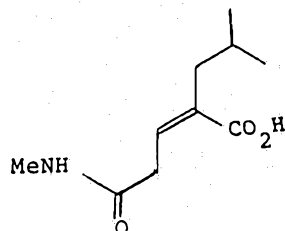
6-Methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-2(and 3)-enoic acids, benzyl esters (D8)



The benzyl ester (D7) (26.46 g, 0.096 mole) in dry acetonitrile (500 ml) under N₂ was cooled to 0° in an ice bath and treated with 1,1'-carbonyldiimidazole (15.69 g, 0.096 mole) in one portion. After 1 h at 0°, a solution of O-methyl-L-tyrosine N-methylamide (20 g, 0.096 mole) in dry acetonitrile (200 ml) was added dropwise with stirring. On completing the addition the mixture was maintained at 0° for 1 h and then allowed to warm to room temperature. Stirring was continued overnight and then solvent was removed in vacuo and the residue taken up in ethyl acetate. After washing with 1N HCl (2x200 ml) and extracting the combined aqueous layers with ethyl acetate (3x100 ml), the combined organic layers were washed with water (100 ml), dried with anhydrous MgSO₄, filtered and evaporated to dryness to give a red oil. Purification by chromatography on silica gel with chloroform followed by 2% methanol/chloroform gave the title compounds as a yellow gum (41.01 g, 92%). Recrystallization of a sample from ethyl acetate/pentane gave a white crystalline product, m.p. 98-101°. (Found: C, 69.38; H, 7.50; N, 5.73. C₂₇H₃₄N₂O₅ requires C, 69.51; H, 7.34; N, 6.00%).

Description 9

4-Methylaminocarbonyl-2-(2-methylpropyl)but-2-enoic acid (D9)



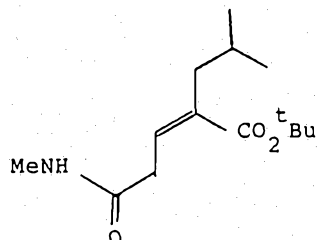
(D9)

A slow stream of anhydrous methylamine was passed into a solution of 2-(2-methylpropyl)pent-2-ene dicarboxylic anhydride (1.3 g) in dry ether (25 ml) at 0°C. After 1 h the methylamine source was removed and the solution was evaporated to dryness in vacuo. The residue was dissolved in ethyl acetate and extracted with 10% aqueous sodium carbonate. The aqueous phase was acidified with dilute hydrochloric acid and re-extracted with ethyl acetate. The extracts were washed with water, dried (MgSO₄), and evaporated to dryness to leave the title compound as a white solid (0.75 g, 49%), m.p. 120-122°C, after trituration with ether; (Found: C, 60.4; H, 8.5; N, 6.95. C₁₀H₁₇NO₃ requires C, 60.3; H, 8.6; N, 7.05%).

δ (d₆-DMSO): 0.85 (6H, d, J=8Hz), 1.75 (1H, m), 2.15 (2H, d, J=6Hz), 2.7 (3H, d, J=5Hz), 3.3 (2H, d, J=8Hz), 6.05 (1H, t, J=8Hz), 7.8 (1H, brs) and 12.4 (1H, brs).

Description 10

4-Methylaminocarbonyl-2-(2-methylpropyl)but-2-enoic acid, tert-butyl ester (D10)



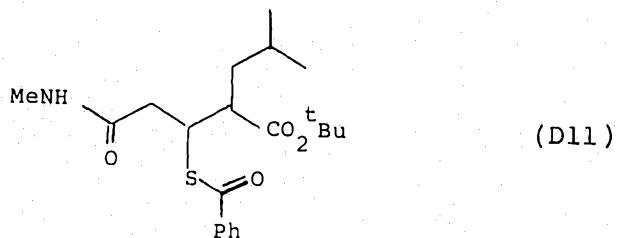
(D10)

A suspension of 4-methylaminocarbonyl-2-(2-methylpropyl)but-2-enoic acid (2.4 g) in chloroform (35 ml) was treated with an excess of isobutylene (30 ml) and concentrated sulphuric acid (3 drops) was added as catalyst. The mixture was stirred at room temperature in a sealed vessel for 8 days. The product was purified by silica gel chromatography [chloroform-pentane (1:1) as eluant] to give the title compound (2.5 g, 81%), m.p. 35-36°C (pentane).

δ (CDCl₃): 0.85 (6H, d, J=8Hz), 1.5 (9H, s), 1.7 (1H, m), 2.1 (2H, d, J=8Hz), 2.8 (2H, d, J=6Hz), 3.2 (2H, d, J=10Hz), 5.9 (1H, t, J=10Hz) and 6.5 (1H, brs).

Description 11

2-(1-Benzoylmercapto-2-[methylaminocarbonyl]ethyl)-4-methylpentanoic acid, tert-butyl ester (D11)

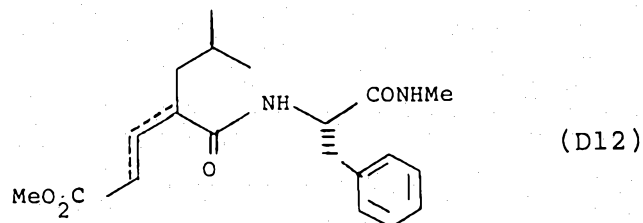


A mixture of 4-methylaminocarbonyl-2-(2-methylpropyl)-but-2-enoic acid, tert-butyl ester (1.25 g, 4.9 mmol) and thiobenzoic acid (1.3 g, 9.4 mmol) was heated at 80°C under nitrogen for 48 h. The product was chromatographed on neutral alumina (50 g) using chloroform as the eluant to give a foam (0.5 g) containing the title compound as the major constituent.

δ (CDCl₃): 0.9 (m), 1.45 (m), 1.65 (m), 2.6 (m), 2.8 (d, J=5Hz), 2.82 (d, J=5Hz), 3.0 (m), 3.2 (m), 4.3 (m), 5.9 (m), 7.4 (m), 7.55 (m) and 7.95 (m). Observed FAB ($M+H$)⁺ 394. C₂₁H₃₁NO₄S requires M 393.

Description 12

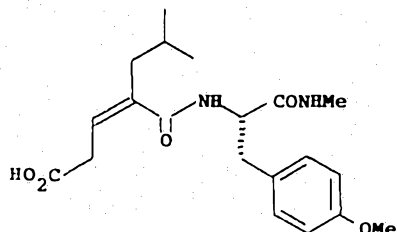
6-Methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-phenyl
ethyl]-amino]carbonyl]hept-2(and 3)-enoic acid, methyl
ester (D12)



Prepared from 4-methoxycarbonyl-2-(2-methylpropyl)but-2-enoic acid (2.0 g, 10 mmol) and L-phenylalanine-N-methylamide (1.87 g, 10.5 mmol) as described for Description 8. The product was chromatographed on silica gel with methanol-dichloromethane (1:24) as the eluant to give the title compound (2.5 g, 69%) as a foam m.p. 78-83°C. (Found: C, 66.7; H, 8.2; N, 7.6. C₂₀H₂₈N₂O₄ requires C, 66.65; H, 7.85; N, 7.75%).

Description 13

6-Methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-3-enoic acid (D13)

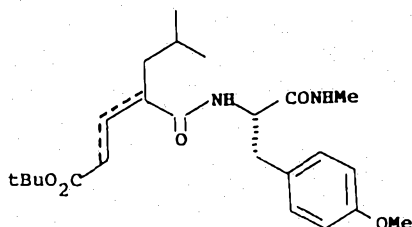


(D13)

A solution of the methyl ester (D6; 7.2 g) in methanol (80 ml) was treated with sodium hydroxide (1.1 g) in water (20 ml) and the solution was stirred at room temperature overnight. The solution was diluted with water, the methanol was removed in vacuo and the aqueous solution was washed with ethyl acetate. The aqueous solution was acidified with 5NHCl and extracted with ethyl acetate (3x50 ml). The combined organic extracts were dried (Na₂SO₄), filtered, and the solvent evaporated in vacuo to give the title compound as a foam (6.6 g).

Description 14

6-Methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-2(and 3)-enoic acid, tert-butyl ester (D14)



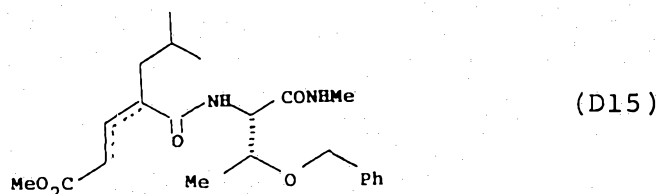
A solution of 6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-3-enoic acid (D13; 6.6 g) in dichloromethane (50 ml) and excess of isobutylene were placed in a sealed vessel with concentrated sulphuric acid for 7 days. After washing with 10% sodium carbonate solution, followed by water, the organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness to give a red oil (4.1 g). Column chromatography on silica gel, eluting with 10% methanol/chloroform gave the title compound as a foam (3.2 g).

(Found: C, 66.51; H, 8.31; N, 6.37. $\text{C}_{24}\text{H}_{36}\text{N}_2\text{O}_5$ requires: C, 66.64; H, 8.39; N, 6.48%)

Observed FAB $(\text{M}+\text{H})^+$ 433 $\text{C}_{24}\text{H}_{36}\text{N}_2\text{O}_5$ requires M 432.

Description 15

6-Methyl-4-[[[2-(R)-benzyloxy-1-(S)-[(methylamino)-
carbonyl]propyl]amino]carbonyl]hept-2(and 3)-enoic
acids, methyl esters (D15)

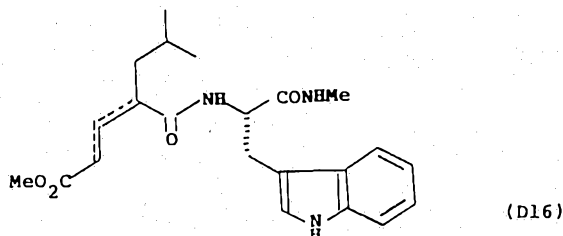


The methyl ester (D5; 3.1 g) in dry acetonitrile (75 ml) under N₂, was cooled to 0° in an ice bath and treated with 1,1'-carbonyldiimidazole (2.74 g) in one portion. After 1 h at 0°, a solution of O-benzyl-L-threonine N-methylamide (3.44 g) in dry acetonitrile (25 ml) was added dropwise. The solution was stirred at room temperature overnight, the solvent removed in vacuo and the residue taken up in ethyl acetate. After washing with 1N HCl (2x25 ml) and extracting the combined aqueous layers with ethyl acetate (3x50 ml), the combined organic layers were washed with water (50 ml), dried (Na₂SO₄), filtered and evaporated to dryness to give an oil. Purification by chromatography on silica gel, eluting with chloroform, followed by 5% methanol/chloroform gave the title compounds as a foam (4.4 g).

(Found: C, 65.51; H, 8.03; N, 6.80. C₂₂H₃₂N₂O₅ requires C, 65.32; H, 7.97; N, 6.92%).

Description 16

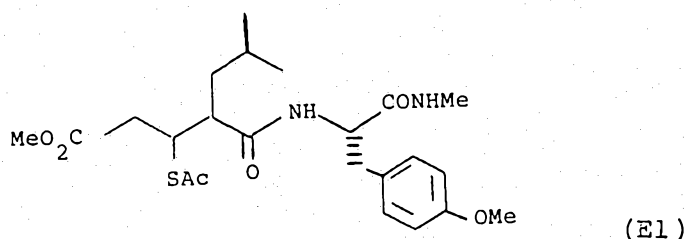
6-Methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(3-indo 1yl)ethyl]amino]carbonyl]hept-2(and 3)-enoic acid, methyl ester (D16)



Prepared from 4-methoxycarbonyl-2-(2-methylpropyl) but-2-enoic acid (1.75 g, 8.75 mmol) and L-tryptophan-N-methylamide (2.64 g, 13 mmol) as described for Description 8. The product was chromatographed on silica gel with methanol-chloroform (1:99) as the eluant to give the title compound (2.8 g, 80%) as a foam m.p. 60-65°C. (Found: C, 66.07; H, 7.37; N, 10.53. $C_{22}H_{29}N_3O_4$ requires C, 66.14; H, 7.32; N, 10.52%).

Example 1

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoic acid, methyl ester (E1)



A solution of 6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-hept-3-enoic acid, methyl ester (D6; 500mg, 1.3mmol) in thiolacetic acid (3ml) was stirred at room temperature for 14 days, then was evaporated to dryness in vacuo. Column chromatography (30g silica) of the residue, eluting with 10 to 25% ethyl acetate/dichloromethane gave the title compound (100mg, 17%) m.p. 118-135°C. Observed mass 466.2137; C₂₃H₂₄N₂O₆S requires 466.2136 Preparative HPLC (Lichrosorb diol), eluting with 20/80 (1% methanol/dichloromethane)/hexane gave two single isomers of the title compound.

Isomer A, mp 162-165°C had the longer retention time. δ (CDCl₃): 0.85 (3H, d, J=7Hz), 0.87 (3H, d, J=7Hz), 1.2-1.7 (m), 2.31 (3H, s), 2.45 (1H, dd, J=8, 17Hz), 2.63 (1H, dd, J=5, 17Hz), 2.6 (1H, m), 2.71 (3H, d, J=5Hz), 2.95 (1H, dd, J=7, 14Hz), 3.03 (1H, dd, J=7, 14Hz), 3.78 (3H, s), 3.66 (3H, s), 3.89 (1H, m), 4.55 (1H, q, J=8Hz), 5.58 (1H, brd), 6.33 (1H, d, J=8Hz), 6.83 (2H, d, J=9Hz) and 7.14 (2H, d, J=9Hz).

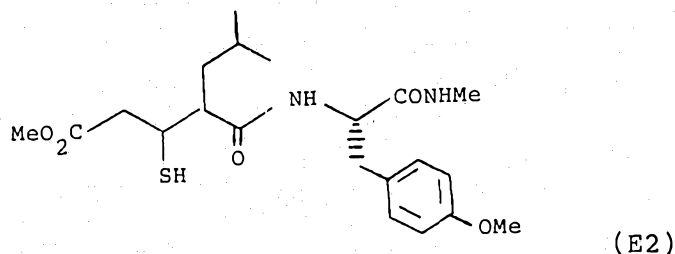
- 40 -

Isomer B, mp 116-118°C had the shorter retention time.

δ (CDCl₃): 0.84 (3H, d, J=7Hz), 0.86 (3H, d, J=7Hz),
1.2-1.7 (m), 2.32 (3H, s), 2.49 (2H, d, J=7Hz), 2.7
(1H, m), 2.72 (3H, d, J=5Hz), 2.97 (1H, dd, J=7, 14Hz),
3.04 (1H, dd, J=7, 14Hz), 3.78 (3H, s), 3.66 (3H, s),
3.97 (1H, m), 4.52 (1H, q, J=8Hz), 5.67 (1H, brs), 6.43
(1H, d, J=8Hz), 6.83 (2H, d, J=9Hz) and 7.15 (2H, d,
J=9Hz).

Example 2

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid methyl ester (E2)



An ice-cooled solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid, methyl ester (E1; 100mg, 0.21mmol) in nitrogen-purged methanol (10ml) was treated with 35% aqueous ammonia (0.5ml), and the reaction mixture was stirred under nitrogen for 20h. The solution was evaporated in vacuo, then was triturated with water, filtered and dried to give the title compound (46mg, 51%), m.p. 80-86°C. Observed mass 424.2028; C₂₁H₃₂O₅N₂S requires 424.2033.

Each of the separated diastereoisomers of the S-acetyl compound of Example 1 (5-15 mg) were individually dissolved in nitrogen-purged methanol (1 ml) and treated with 35% aqueous ammonia (0.1 ml). The solutions were stirred at room temperature under nitrogen for 2 h, then were evaporated in vacuo, and the residues were triturated with water and dried to give:

Isomer A, m.p. 193-196°C:

δ (CDCl₃): 0.87 (6H, d, J=6Hz), 1.2-1.7 (3H, m), 1.67 (1H, d, J=9Hz), 2.32 (1H, dd, J=10, 15Hz), 2.41 (1H, m), 2.59 (1H, dd, J=4, 15Hz), 2.73 (3H, d, J=5Hz), 3.0 (2H, d, J=8Hz), 3.21 (1H, m), 3.70 (3H, s), 3.78 (3H, s), 4.59 (1H, q, J=7Hz), 5.66 (1H, brd), 6.34 (1H, d, J=8Hz), 6.83 (2H, d, J=9Hz) and 7.13 (2H, d, J=9Hz).

Observed mass. 424.2039; C₂₁H₃₂N₂O₅S requires 424.2031

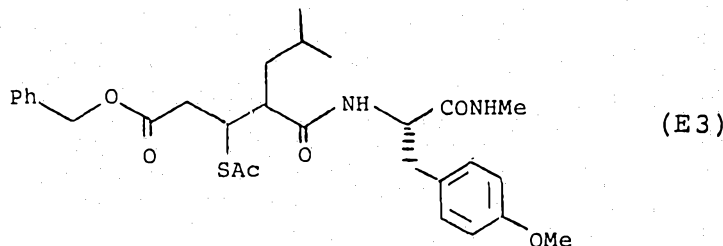
and Isomer B:

δ (CDCl₃): 0.86 (6H, t, J=7Hz), 1.2-1.7 (3H, m), 2.00 (1H, d, J=9Hz), 2.49 (1H, m), 2.51 (1H, dd, J=9, 15Hz), 2.68 (1H, dd, J=5, 16 Hz), 2.73 (3H, d, J=5Hz), 2.97 (1H, dd, J=7, 13Hz), 3.07 (1H, dd, J=7H, 13Hz), 3.28 (1H, m), 3.72 (3H, s), 3.79 (3H, s), 4.55 (1H, q, J=7Hz), 5.64 (1H, brs), 6.33 (1H, d, J=7Hz), 6.84 (2H, d, J=8Hz) and 7.14 (2H, d, J=7Hz).

Observed mass. 424.2024; C₂₁H₃₂N₂O₅S requires 424.2031.

Example 3

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoic acid, benzyl ester (E3)



The benzyl ester (D8) (41g, 0.088 mole) was dissolved in thiolacetic acid (170 ml) and left to stand at room temperature under nitrogen for 19 days. Volatile material was removed in vacuo and the residue chromatographed on silica gel with initially ether, rising slowly to ethyl acetate. After removal of high R_f impurities the title compound was obtained as a mixture of diastereomers as a pale yellow foam (29.76g, 62.4%). (Found: C, 64.29; H, 7.16; N, 4.86. $C_{29}H_{38}N_2SO_6$ requires C, 64.18; H, 7.06; N, 5.16%). Later fractions contained olefinic material (D8). However, more complete reaction can be obtained by addition of a suitable base, for example triethylamine. An aliquot (2 ml) was removed from the above reaction mixture after 14 days, treated with triethylamine (3 drops) and left to stand for a further 5 days at room temperature under nitrogen. The same work-up procedure as for the bulk of the reaction mixture described above gave only the diastereomer mixture (E3) with no olefinic material remaining.

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid, benzyl ester (E4, Isomer A, 2.0 g 0.004 mol) was dissolved in chloroform (100 ml) under an atmosphere of nitrogen. The reaction mixture was cooled to 0°C and acetic anhydride (2.04 g, 0.02 mol) was added followed by N-methylmorpholine (2.02 g, 0.02 mol). The reaction mixture was allowed to warm to room temperature, then stirred under nitrogen for 3 days.

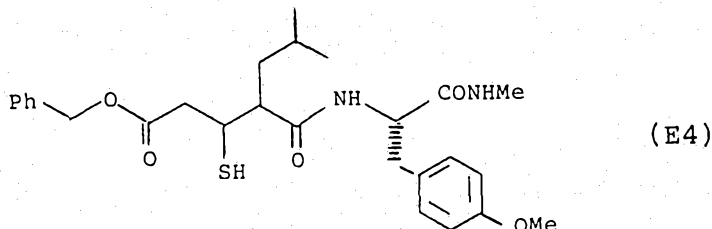
The chloroform solution was washed with 10% aqueous citric acid solution then water, then dried (MgSO₄) and concentrated in vacuo to give a white solid which was recrystallised from ether to give the title compound as a single isomer (Isomer A) (1.4 g), m.p. 89-90°C.

(Found: C, 63.94; H, 7.25; N, 5.13. C₂₉H₃₈N₂O₆S requires C, 64.18; H, 7.06; N, 5.16%.

δ (CDCl₃): 0.84(6H, t, J=7Hz), 1.17-1.71(3H, m), 2.27(3H, s), 2.45-2.7(3H, m), 2.7(3H, d, J=5Hz), 2.85-3.04(2H, m), 3.78(3H, s), 3.92(1H, m), 4.52(1H, q, J=8Hz), 5.12(2H, s), 5.52(1H, brd), 6.32(1H, d, J=8Hz), 6.79(2H, d, J=9Hz), 7.12(2H, d, J=9Hz) and 7.36(5H, m).

Example 4

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid, benzyl ester (E4)



An ice-cooled solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-amino]carbonyl]heptanoic acid, benzyl ester (E3; 1 g, 2 mmol) in nitrogen-purged methanol (100 ml) was treated with 35% aqueous ammonia (10 ml). The mixture was stirred at room temperature under nitrogen for 3 h and the precipitated solid (100 mg) was filtered off. The filtrate was evaporated in vacuo, and the residue was crystallised from methanol (5 ml) and ether (120 ml), to give a single isomer (isomer A) of the title compound (300 mg), m.p. 199-201°C.

(Found: C, 64.77; H, 7.31; N, 5.68. $C_{27}H_{36}N_2O_5S$ requires C, 64.77; H, 7.25; N, 5.60%).

δ (CDCl₃): 0.84 (3H, d, J=7Hz), 0.86 (3H, d, J=7Hz), 1.3-1.7 (3H, m), 1.66 (1H, d, J=9Hz), 2.38 (1H, dd, J=9, 15Hz), 2.41 (1H, m), 2.66 (1H, dd, J=4, 15Hz), 2.73 (3H, d, J=5Hz), 2.97 (2H, d, J=8Hz), 3.22 (1H, m), 3.74 (3H, s), 4.57 (1H, q, J=8Hz), 5.14 (2H, s), 5.63 (1H, brd), 6.33 (1H, d, J=8Hz), 6.81 (2H, d, J=9Hz), 7.12 (2H, d, J=9Hz) and 7.36 (5H, m).

Column chromatography of the mother liquors on silica, eluting with 3:1 ether:chloroform gave two further single diastereoisomers.

Isomer B, the slower running isomer was obtained as a foam, which solidified on standing, mp 133-135°C. (Found: C, 64.66; H, 7.41; N, 5.58. $C_{27}H_{36}N_2O_5S$ requires C, 64.77; H, 7.25; N, 5.60%).

δ ($CDCl_3$): 0.73 (3H, d, J=7Hz), 0.79 (3H, d, J=7Hz), 1.2-1.7 (3H, m), 1.90 (1H, d, J=9Hz), 2.36 (1H, m), 2.64 (1H, dd, J=9, 17Hz), 2.69 (3H, d, J=6Hz), 2.81 (1H, dd, J=4, 17Hz), 2.94 (1H, dd, J=7, 16Hz), 3.03 (1H, dd, J=7, 16Hz), 3.26 (1H, m), 3.78 (3H, s), 4.59 (1H, q, J=7Hz), 5.15 (2H, m), 5.98 (1H, brd), 6.16 (1H, d, J=8Hz), 6.82 (2H, d, J=9Hz), 7.10 (2H, d, J=9Hz) and 7.36 (5H, m).

Isomer C, the faster running isomer was obtained as a gum.

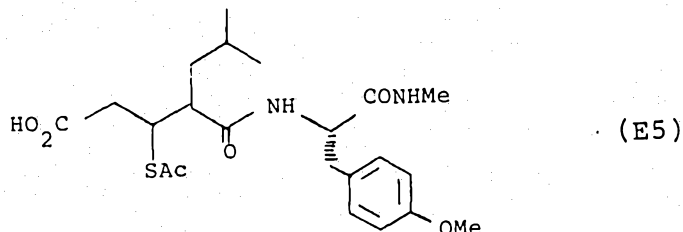
δ ($CDCl_3$): 0.81 (3H, d, J=6Hz), 0.83 (3H, d, J=6Hz), 1.2-1.7 (3H, m), 1.99 (1H, d, J=9Hz), 2.47 (1H, m), 2.56 (1H, dd, J=8, 15Hz), 2.72 (1H, m), 2.72 (3H, d, J=6Hz), 2.96 (1H, dd, J=7, 15Hz), 3.04 (1H, dd, J=7, 15Hz), 3.29 (1H, m), 3.77 (3H, s), 4.55 (1H, q, J=7Hz), 5.16 (2H, s), 5.75 (1H, brd), 6.38 (1H, d, J=8Hz), 6.81 (2H, d, J=9Hz), 7.12 (2H, d, J=9Hz) and 7.36 (5H, m).

Isomer D was obtained from a mixture of isomers B, and D by preparative HPLC (Lichrosorb diol), eluting with 18/82 (1% methanol/dichloromethane)/hexane.

δ ($CDCl_3$): 0.68 (3H, dd, J=7Hz), 0.78 (3H, d, J=7Hz), 1.1-1.6 (3H, m), 2.09 (1H, d, J=10Hz), 2.36 (1H, m), 2.63 (1H, dd, J=6, 17Hz), 2.74 (3H, d, J=6Hz), 2.85 (1H, dd, J=5, 17Hz), 2.99 (1H, dd, J=7, 16Hz), 3.09 (1H, dd, J=7, 16Hz), 3.29 (1H, m), 3.79 (3H, s), 4.68 (1H, q, J=7Hz), 5.91 (1H, d, J=8Hz), 6.24 (1H, brd), 6.82 (2H, d, J=9Hz) and 7.10 (2H, d, J=9Hz).

Example 5

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoic acid (E5)



A solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid, benzyl ester (E3; 300 mg, 0.55 mmol) in 4.5% formic acid/methanol (6 ml) was added, under nitrogen, to a stirred suspension of palladium black (310 mg) in the same solvent (15 ml). After 90 min the mixture was filtered through kieselguhr and evaporated in vacuo. Column chromatography using reverse phase silica (30 g) and eluting with 25% aqueous methanol gave two single diastereoisomers of the title compound, as monohydrates.

Isomer A, m.p. 92-95°C (54 mg).

(Found: C, 56.34; H, 7.11; N, 6.08. $C_{22}H_{32}N_2O_6S \cdot H_2O$ requires C, 56.15; H, 7.28; N, 5.95%).

δ (CDCl₃): 0.85 (3H, d, J=6Hz), 0.91 (3H, d, J=6Hz), 1.2-1.7 (3H, m), 2.31 (3H, s), 2.50 (1H, dd, J=3, 17Hz), 2.67 (1H, dd, J=7, 17Hz), 2.68 (3H, d, J=5Hz), 2.85-3.0 (2H, m), 3.15 (1H, t, J=10Hz), 3.74 (3H, s), 3.80 (1H, m), 4.80 (1H, q, J=8Hz), 6.36 (1H, m), 6.77 (2H, d, J=9Hz), 7.07 (2H, d, J=9Hz) and 8.41 (1H, d, J=10Hz).

and Isomer B, m.p. 106-110°C (70 mg).

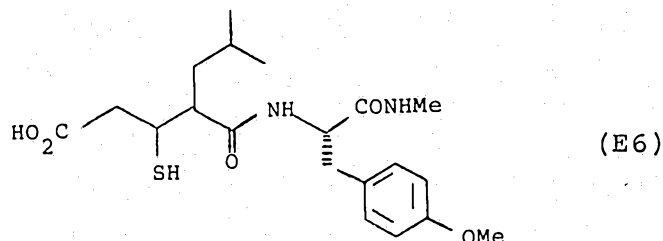
(Found: C, 56.33; H, 7.17; N, 6.15. $C_{22}H_{32}N_2O_6S \cdot H_2O$ requires C, 56.15; H, 7.28; N, 5.95%).

δ ($CDCl_3$): 0.77 (3H, s), 0.86 (3H, s), 1.1-1.7 (3H, m), 2.34 (3H, s), 2.73 (2H, d, $J=4Hz$), 2.82 (3H, d, $J=4Hz$), 2.8-3.0 (3H, m), 3.75 (3H, s), 3.88 (1H, brd, $J=11Hz$), 4.80 (1H, q, $J=8Hz$), 6.61 (1H, m), 6.78 (2H, d, $J=9Hz$), 7.11 (2H, d, $J=9Hz$) and 8.53 (1H, d, $J=9Hz$).

Similar treatment of the single isomer of the acetylmercapto compound (E3, Isomer A; 1.4 g, 2.6 mmol) gave the title compound as a single isomer (Isomer A), 980 mg (84%).

Example 6

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-
2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid
(E6)



Each of the separate isomers of the S-acetyl compound of Example 5 (27-42 mg) were individually dissolved in nitrogen-purged methanol (4 ml) and treated with 35% aqueous ammonia (0.4 ml). The solutions were stirred at room temperature under nitrogen for 6 h, then were evaporated in vacuo, and the residues were triturated with ether to give:

Isomer A, m.p. 171-176°C:

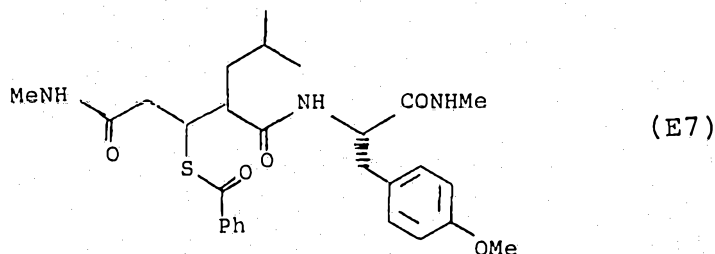
δ (CDCl₃): 0.86 (6H, t, J=6Hz), 1.3-1.7 (3H, m), 1.79 (1H, d, J=9Hz), 2.43 (1H, dd, J=5, 15Hz), 2.66 (1H, dd, J=4, 15Hz), 2.72 (3H, d, J=5Hz), 2.8-3.1 (4H, m), 3.74 (3H, s), 4.73 (1H, q, J=7Hz), 6.29 (1H, brs), 6.89 (2H, d, J=9Hz), 7.09 (2H, d, J=9Hz) and 8.02 (1H, brd, J=9Hz).

and Isomer B, m.p. 107-110°C:

δ (CDCl₃): 0.76 (3H, d, J=7Hz), 0.79 (3H, d, J=7Hz), 2.34 (1H, d, J=10Hz), 2.73 (2H, d, J=6Hz), 2.80 (3H, d, J=6Hz), 2.85-3.2 (4H, m), 3.77 (3H, s), 4.71 (1H, q, J=8Hz), 6.26 (1H, brs), 6.79 (2H, d, J=9Hz), 7.09 (2H, J=9Hz) and 7.94 (1H, brd, J=9Hz).

Example 7

3-Benzoylmercapto-N⁵-methyl-N¹-[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)-
pentanediamide (E7)



2-(1-Benzoylmercapto-2-[methylaminocarbonyl]ethyl)-4-methylpentanoic acid, tert-butyl ester (D11; 0.5 g) was treated with trifluoroacetic acid (10 ml) at 0°C for 2h and evaporated to dryness in vacuo to give 2-(1-benzoylmercapto-2-[methylaminocarbonyl]ethyl)-4-methylpentanoic acid.

[δ (CDCl₃) no signal at 1.5 ppm indicating absence of a tert-butyl group]

N,N-Dicyclohexylcarbodiimide (235 mg) and hydroxybenzotriazole (145 mg) was added to an ice-cooled solution of the above acid and O-methyl-L-tyrosine-N-methylamide (220 mg) in dry dichloromethane (20 ml), and the mixture was stirred at room temperature for 18 h. The precipitated solid was filtered off, and washed with a little dichloromethane, then the organic solution was washed successively with 1N hydrochloric acid, 1N sodium bicarbonate and water. The solution was dried (MgSO₄) and evaporated to dryness in vacuo. The residual solid was chromatographed on neutral alumina using chloroform-pentane (1:1) as eluant. The solid product was triturated with ether to give the title

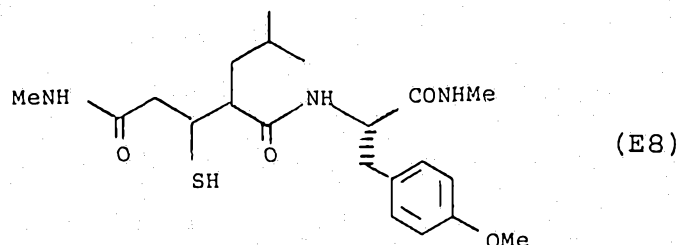
- 51 -

compound (130 mg), m.p. 140-144°C as a mixture of diastereoisomers.

Observed M^+ 527.2449. $C_{28}H_{37}N_3O_5S$ requires M 527.2453.

Example 8

3-Mercapto-N⁵-methyl-N¹-[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)pentane-
diamide (E8)



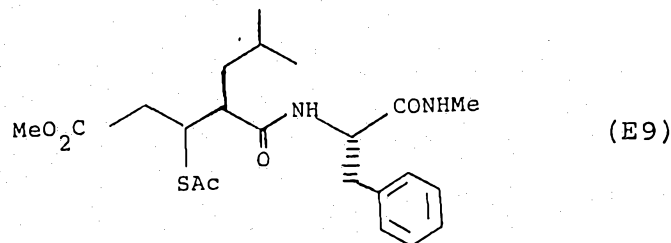
An ice-cooled solution of 3-benzoylmercapto-N⁵-methyl-N¹-[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)pentanediamide (E7; 40 mg) in nitrogen-purged methanol (5 ml) was treated with 35% aqueous ammonia (2.5 ml) and the reaction mixture was stirred under nitrogen for 2 h. The solution was filtered and the filtrate was evaporated to dryness in vacuo. The residue was triturated with ether, filtered, and dried to give the title compound (20 mg, 62%), m.p. 175-190°C as a mixture of diastereoisomers. Observed M⁺ 423.2190. C₂₁H₃₃N₃O₄S requires M 423.2191.

Similar treatment of 3-acetylmercapto-N⁵-methyl-N¹-(1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)-ethyl-2-(2-methylpropyl)pentanediamide (E11, Isomer A; 48 mg, 0.1 mmol) gave the title compound as a single isomer (Isomer A), (28 mg, 64%), mp 243-250°C. (Found: C, 59.89; H, 7.86; N, 9.55. C₂₁H₃₃N₃O₄S requires C, 59.55; H, 7.85; N, 9.92%).

δ (CDCl₃ + drop d₆-DMSO): 0.83 (3H, d, J=8Hz), 0.85 (3H, d, J=8Hz), 1.2-1.6 (3H, m), 1.74 (1H, d, J=7Hz), 2.0 (1H, dd, J=11, 13Hz), 2.10 (1H, dd, J=3, 13Hz), 2.65 (6H, d, J=5Hz), 2.79 (1H, dd, J=9, 13Hz), 2.98 (1H, dd, J=5, 13Hz), 3.73 (3H, s), 4.56 (1H, m), 6.87 (2H, d, J=9Hz), 7.15 (2H, d, J=9Hz), 7.55 (2H, m) and 7.98 (1H, d, J=9Hz).

Example 9

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-phenylethyl]amino]carbonyl]-heptanoic acid,
methyl ester (E9)



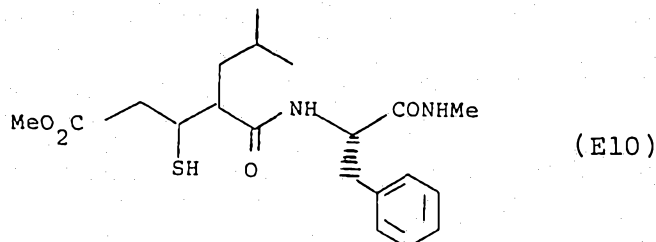
A solution of 6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-phenylethyl]amino]carbonyl]-hept-3-enoic acid, methyl ester (D12; 2.3 g, 6.3 mmol) in thiolacetic acid (20 ml) was stirred at room temperature for 14 days, then was evaporated to dryness in vacuo. The product was chromatographed on silica gel (100 g) eluting with 10 to 25% ethyl acetate/pentane, to give the title compound (1.35 g, 49%) (mixture of diastereoisomers).

The product was recrystallised (x2) from ethyl acetate to give a solid (0.7 g) m.p. 188-189°C which was shown by NMR to be a mixture of two diastereoisomers (ratio 3:1). (Found: C, 60.9; H, 7.3; N, 6.35. $C_{22}H_{32}N_2O_5S$ requires C, 60.55; H, 7.45; N, 6.4%).

δ ($CDCl_3$): 0.85 (6H, m), 1.2-1.7 (3H, m), 2.3 (s) and 2.32 (s) (total 3H), 2.4 (1H, m), 2.6 (2H, m), 2.71 (3H, d, $J=6$ Hz), 3.07 (2H, m), 3.66 (s) and 3.68 (s) (ratio 3:1 - total 3H), 3.9 (1H, m), 4.65 (1H, m), 5.78 (brd) and 5.83 (brd) (ratio 3:1 - total 1H), 6.43 (d, $J=8$ Hz) and 6.51 (d, $J=8$ Hz) (ratio 3:1 - total 1H) and 7.25 (5H, m).

Example 10

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-phenylethyl]amino]carbonyl]heptanoic acid, methyl ester (E10)



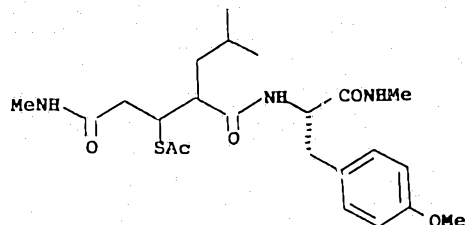
An ice-cooled solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-phenylethyl]amino]carbonyl]heptanoic acid, methyl ester, m.p. 188-189°C, (E9; 100 mg) in nitrogen-purged methanol (5 ml) was treated with 35% aqueous ammonia (2 ml) and the reaction mixture was stirred under nitrogen for 3.5 h. The solution was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was triturated with water and the solid was collected and recrystallised from ethyl acetate-ether to give the title compound (25 mg), m.p. 169-171°C, as a single diastereoisomer (Isomer A).

(Found: C, 60.65; H, 7.85; N, 7.05. $C_{20}H_{30}N_2O_4S$ requires C, 60.9; H, 7.65; N, 7.1%).

δ ($CDCl_3$): 0.85 (6H, d, $J=5\text{Hz}$), 1.2-1.7 (3H, m), 1.64 (1H, d, $J=9\text{Hz}$), 2.3 (1H, dd, $J=9, 16\text{Hz}$), 2.42 (1H, m), 2.58 (1H, dd, $J=4, 16\text{Hz}$), 3.07 (1H, d, $J=8\text{Hz}$), 3.2 (1H, m), 3.68 (3H, s), 4.65 (1H, q, $J=8\text{Hz}$), 5.75 (1H, brd), 6.39 (1H, d, $J=8\text{Hz}$) and 7.25 (5H, m).

Example 11

3-Acetylmercapto-N⁵-methyl-N¹-[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methyl-
propyl)pentanediamide (E11)



(E11)

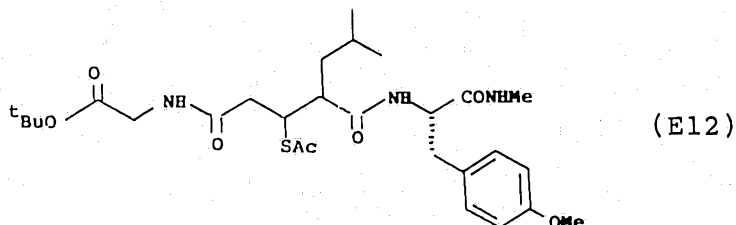
Diisopropylethylamine (0.13 ml, 0.7 mmol) and ethyl chloroformate (0.07 ml, 0.7 mmol) were added to a solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid (E5, Isomer A; 300 mg, 0.66 mmol) in dry THF (10 ml), at -20°C. After 15 min a solution of methylamine (20 mg) in THF (0.8 ml) was added dropwise and the mixture was stirred in an ice bath for 3 h. The solvent was evaporated in vacuo, and a solution of the residue in dichloromethane was washed successively with water, 1M hydrochloric acid, water, saturated sodium hydrogen carbonate and brine. The solution was dried (MgSO₄) and evaporated in vacuo. Column chromatography on silica (10 g), eluting with 5% methanol/ethyl acetate gave the title compound, mp 195-199°C.

(Found: C, 58.97; H, 7.78; N, 8.92. C₂₃H₃₅N₃O₅S requires C, 59.33; H, 7.58; N, 9.03%).

δ (CDCl₃): 0.84 (6H, q, J=3Hz), 1.2-1.7 (3H, m), 2.10 (1H, dd, J=7, 15Hz), 2.30 (3H, s), 2.31 (1H, dd, J=5, 15Hz), 2.68 (1H, m), 2.74 (3H, d, J=5Hz), 2.76 (3H, d, J=5Hz), 2.95 (1H, dd, J=6, 14Hz), 3.08 (1H, dd, J=6, 14Hz), 3.78 (3H, s), 3.83 (1H, m), 4.63 (1H, q, J=7Hz), 5.49 (1H, brs), 5.88 (1H, brs), 6.68 (1H, d, J=7Hz), 6.82 (2H, d, J=8Hz) and 7.14 (2H, d, J=8Hz).

Example 12

2-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoylamino]ethanoic acid, t-butyl ester (E12).



A solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid (E5, Isomer A; 90 mg, 0.2 mmol), N-ethyl-N'-dimethylaminopropylcarbodiimide hydrochloride (42 mg, 0.22 mmol), 1-hydroxybenzotriazole (34 mg, 0.22 mmol), t-butylglycine hydrochloride (37 mg, 0.22 mmol) and diisopropylethylamine (0.07 ml, 0.4 mmol) in dichloromethane (2 ml), was stirred at room temperature for 18 h. The mixture was diluted with dichloromethane and was washed successively with saturated sodium hydrogen carbonate, water, 1M citric acid and brine. The solution was dried (NaSO₄) and evaporated in vacuo to leave the title compound as a single isomer (Isomer A) (74 mg, 68%), mp 192-194°C (ethyl acetate).

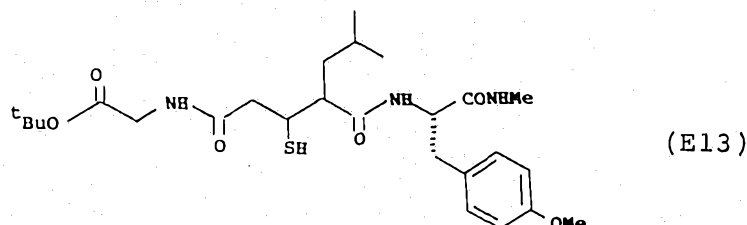
- 58 -

(Found: C, 59.51; H, 7.70; N, 7.33. $C_{28}H_{43}N_3O_7S$ requires C, 59.45; H, 7.66; N, 7.43%).

δ ($CDCl_3$): 0.77 (3H, d, $J=6$ Hz), 0.83 (3H, d, $J=6$ Hz), 1.2-1.7 (3H, m), 1.47 (9H, s), 2.30 (3H, s), 2.35 (2H, m), 2.56 (1H, m), 2.77 (3H, d, $J=5$ Hz), 3.00 (1H, dd, $J=10, 15$ Hz), 3.10 (1H, dd, $J=7, 15$ Hz), 3.70 (1H, dd, $J=6, 19$ Hz), 3.78 (1H, s), 3.95 (1H, dd, $J=6, 19$ Hz), 4.00 (1H, m), 4.64 (1H, q, $J=7$ Hz), 6.05 (1H, m), 6.33 (1H, brt), 6.83 (2H, d, $J=9$ Hz), 7.14 (2H, d, $J=9$ Hz) and 7.43 (1H, d, $J=7$ Hz).

Example 13

2-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoylamino]ethanoic acid, t-butyl ester (E13)



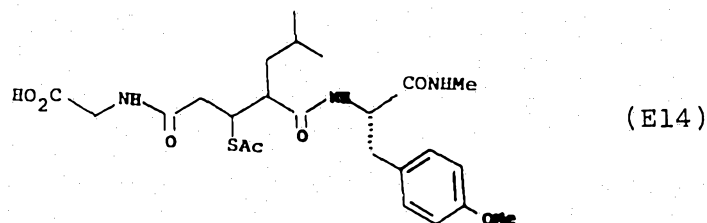
An ice-cooled solution of 2-[3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoylamino]ethanoic acid, t-butyl ester (E12, Isomer A; 30 mg, 0.053 mmol) in nitrogen-purged methanol (4 ml) was treated with 35% aqueous ammonia (0.3 ml), under nitrogen, and was stirred for 2 h at room temperature. The solvents were evaporated in vacuo and the residue was triturated with hexane to give the title compound as a single isomer (Isomer A), mp 223-226°C.

(Found: C, 59.52; H, 7.68; N, 7.88. $C_{26}H_{41}N_3O_6S$ requires C, 59.63; H, 7.89; N, 8.02%).

δ ($CDCl_3$): 0.84 (6H, dd, $J=6, 8$ Hz), 1.3-1.7 (3H, m), 1.48 (9H, s), 2.24 (1H, d, $J=10$ Hz), 2.28 (1H, m), 2.45 (1H, dd, $J=15, 5$ Hz), 2.50 (1H, m), 2.76 (3H, d, $J=5$ Hz), 2.99 (1H, dd, $J=15, 9$ Hz), 3.09 (1H, dd, $J=15, 7$ Hz), 3.12 (1H, m), 3.77 (3H, s), 3.89 (1H, dd, $J=5, 7$ Hz), 4.64 (1H, q, $J=8$ Hz), 5.98 (1H, brd), 6.47 (1H, brt), 6.84 (2H, d, $J=9$ Hz), 7.15 (2H, d, $J=9$ Hz) and 7.18 (1H, brs).

Example 14

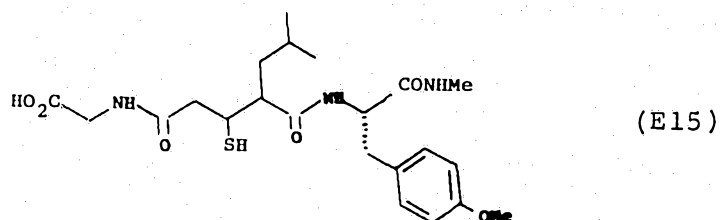
2-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-
[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoylamino]ethanoic acid (E14)



A solution of 2-[3-acetylmercapto-6-methyl-4-[[[1-(S)-
 [(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
 amino]carbonyl]heptanoylamino]ethanoic acid, t-butyl
 ester (E12, Isomer A; 60 mg, 0.11 mmol) in
 trifluoroacetic acid (1.5 ml) and water (0.05 ml) was
 stirred in an ice bath for 1 h, then at room
 temperature for 2 h. The solvent was evaporated in
vacuo and the residue was azeotroped dry with toluene
 to leave the title compound as a single isomer (Isomer
 A), mp 188-192°C.

Example 15

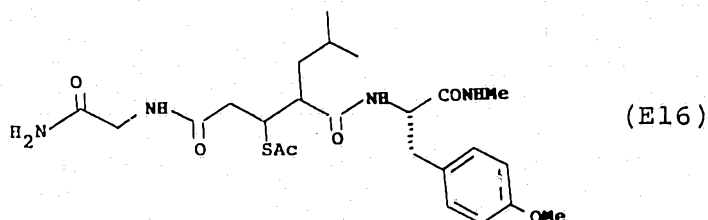
2-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoylamino]ethanoic acid (E15)



A solution of 2-[3-acetylmercapto-6-methyl-4-[[[1-(S)-
[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoylamino]ethanoic acid (E14,
Isomer A; 15 mg, 0.029 mmol) in nitrogen-purged
methanol (5 ml) was cooled in ice and treated with 35%
aqueous ammonia (0.3 ml). After 2 h the solvents were
evaporated in vacuo and the residue was triturated with
ethyl acetate to give the title compound as a single
isomer (Isomer A), 11 mg (80%), mp 228-231°C.

Example 16

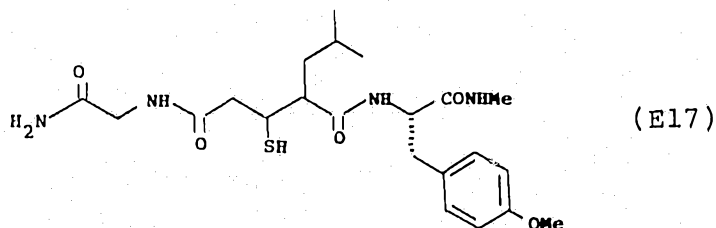
3-Acetylmercapto-N⁵-(aminocarbonyl)methyl-N¹-[1-(S)-
[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-
ethylpropyl)pentanediamide (E16)



A mixture of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-amino]carbonyl]heptanoic acid (E5, Isomer A; 226 mg, 0.5 mmol), N-ethyl-N¹-dimethylaminopropylcarbodiimide hydrochloride (116 mg, 0.6 mmol), 1-hydroxybenzotriazole (90 mg, 0.58 mmol), glycineamide hydrochloride (64 mg, 0.58 mmol) and diisopropylethylamine (0.2 ml, 1.14 mmol) in dichloromethane was stirred at 5°C for 2 h then at room temperature overnight. The mixture was cooled in ice and the solid was collected by filtration and washed well with dichloromethane. The solid was triturated with 1M citric acid, then filtered, washed with water and dried. Recrystallisation from methanol/ ether gave the title compound as a single isomer (Isomer A), 75 mg (30%) mp 218-219°C.

δ (d₆-DMSO): 0.78(6H,t,J=7Hz), 1.1-1.5(3H,m), 2.1-2.3(2H,m), 2.24(1H,s), 2.55(3H,d,J=5Hz), 2.74(1H,dd,J=10,14Hz), 2.85(1H,dd,J=5,14Hz), 3.56(2H,d,J=6Hz), 3.68(3H,s), 3.77(1H,m), 4.43(1H,m), 6.81(2H,d,J=9Hz), 7.10(2H,brd), 7.14(2H,d,J=9Hz), 7.79(1H,brq), 8.00(1H,t,J=6Hz) and 8.12(1H,d,J=8Hz).

N⁵-(Aminocarbonyl)methyl-3-mercapto-N¹-[1-(S)-
[(methyl amino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-
(2-methylpropyl)pentanediamide (E17)

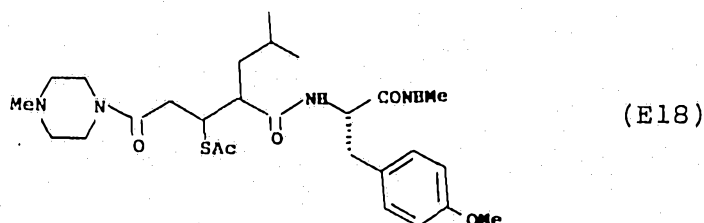


An ice-cooled solution of 3-acetylmercapto-N⁵-(amino-carbonyl)methyl-N¹-[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)pentanediamide (E16, Isomer A; 44 mg, 0.087 mmol) in nitrogen-purged methanol (5 ml) was treated with 35% aqueous ammonia (0.4 ml) and was stirred at room temperature for 2.5h. The solvent was evaporated in vacuo and the residue was triturated with ether to leave the title compound as a single isomer (Isomer A), 28 mg (70%), mp 247-252°C.

δ (d_6 -DMSO): 0.77(3H,d,J=6Hz), 0.82(3H,d,J=6Hz), 1.2-1.5(3H,m), 2.04(2H,m), 2.30(1H,d,J=7Hz), 2.33(1H,m), 2.57(3H,d,J=5Hz), 2.70(1H,d,J=10,14Hz), 2.86(1H,dd,J=5,14Hz), 3.08(1H,m), 3.62(2H,q,J=5Hz), 3.67(3H,s), 4.46(1H,m), 6.80(2H,d,J=9Hz), 7.10(2H,brd), 7.15(2H,d,J=9Hz), 7.81(1H,m), 8.06(1H,t,J=5Hz) and 8.18(1H,d,J=8Hz).

Example 18

1-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoyl]-4-methylpiperazine (E18)



A mixture of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]-carbonyl]heptanoic acid (E5, Isomer A; 226 mg, 0.5 mmol), *N,N*-dicyclohexylcarbodiimide (103 mg, 0.5 mmol), 1-hydroxybenzotriazole (76 mg, 0.5 mmol) and *N*-methylpiperazine (0.05 ml, 0.5 mmol) in dichloromethane (8 ml) was stirred at 5°C for 1 h, then at room temperature overnight. After cooling, the precipitated solid was filtered off. The solution was diluted with dichloromethane and extracted with 0.25 M citric acid. The extracts were washed with dichloromethane then basified with 10% sodium carbonate, and extracted with ether. The ether extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo. Trituration of the oily residue with ether, followed by recrystallisation of the resulting solid from ethyl acetate/hexane gave the title compound as a single isomer (Isomer A), 98 mg (37%) mp 133-138°C.

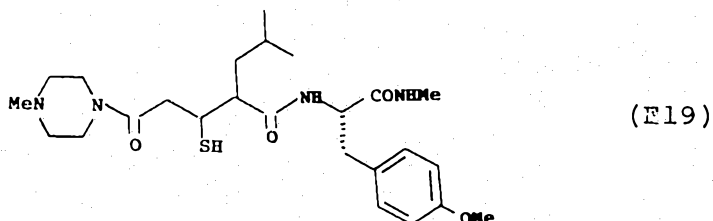
- 65 -

(Found: C, 60.48; H, 8.15; N, 10.40. $C_{27}H_{42}N_4O_4S$ requires
C, 60.65; H, 7.92; N, 10.48%).

δ ($CDCl_3$): 0.85 (6H, q, $J=3\text{Hz}$), 1.2-1.5 (3H, m), 2.30 (6H, s),
2.38 (4H, brs), 2.33-2.8 (3H, m), 2.70 (3H, d, $J=5\text{Hz}$), 2.98
(2H, d, $J=7\text{Hz}$), 3.4 (2H, brs), 3.5-3.7 (2H, brm), 3.78 (3H, s),
3.95 (1H, q, $J=6\text{Hz}$), 4.54 (1H, q, $J=7\text{Hz}$), 5.71 (1H, brd),
6.64 (1H, brd), 6.82 (2H, d, $J=9\text{Hz}$) and 7.15 (2H, d, $J=9\text{Hz}$).

Example 19

1-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbon yl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoyl]-4-methylpiperazine (E19)

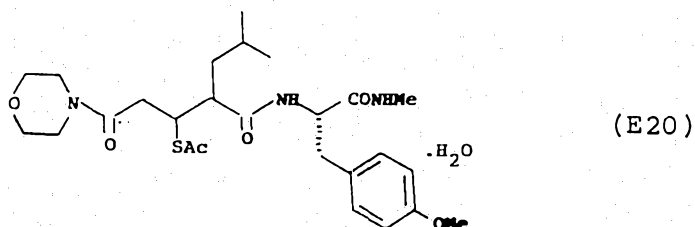


A solution of 1-[3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]-carbonyl]heptanoyl]-4-methylpiperazine (E18, Isomer A; 60 mg, 0.112 mmol) in methanol (5 ml) was purged with nitrogen and cooled in ice, then was treated with 35% aqueous ammonia (0.4 ml). After 6 h at room temperature, the solvents were evaporated in vacuo and the residue was triturated with hexane to give the title compound as a single isomer (Isomer A), mp 65-68°C.

δ (CDCl₃): 0.87 (6H, d, J=6Hz), 1.3-1.7 (3H, m), 1.85 (1H, d, J=8Hz), 2.35 (7H, m), 2.42 (1H, dd, J=8, 16Hz), 2.54 (1H, m), 2.61 (1H, dd, J=4, 16Hz), 2.74 (3H, d, J=5Hz), 3.01 (2H, d, J=8Hz), 3.30 (1H, m), 3.48 (2H, brs), 3.65 (2H, brm), 3.79 (3H, s), 4.58 (1H, q, J=8Hz), 5.35 (1H, brm), 5.74 (1H, brd), 6.52 (1H, d, J=8Hz), 6.82 (2H, d, J=9Hz) and 7.15 (2H, d, J=9Hz).

Example 20

1-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoyl]morpholine hydrate (E20)



To a solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-amino]carbonyl]heptanoic acid (E5, Isomer A; 290 mg, 0.64 mmol) in acetonitrile (10 ml) at 0°C was added 1,1'-carbonyl diimidazole (104 mg, 0.64 mmol) and the solution was stirred under nitrogen at 0°C for 1 h. A solution of morpholine ((56 mg, 0.64 mmol) in acetonitrile (2 ml) was then added dropwise, and the solution was left at room temperature overnight. The mixture was evaporated to dryness in vacuo and then dissolved in a mixture of ethyl acetate and chloroform (4:1; 30 ml) and washed successively with sodium carbonate, 1M-hydrochloric acid and water. The organic fraction was dried (MgSO₄) and chromatographed on silica gel 60 (100 g). Elution with 5% methanol-ethyl acetate gave the title compound (70 mg) as a single isomer (Isomer A), m.p. 146-149°C, after trituration with ether.

(Found: C, 57.73; H, 7.64; N, 7.59. $C_{25}H_{39}N_3O_6S \cdot H_2O$

requires C, 57.86; H, 7.66; N, 7.79%).

δ ($CDCl_3$): 0.86(d, J=Hz) and 0.87(d, J=5Hz) (total 6H),

1.2-1.7(2H, m), 2.32(3H, s), 2.48(1H, dd, J=4, 16Hz),

2.62(1H, dd, J=4, 16Hz), 2.72(4H, brd, J=5Hz),

2.98(2H, d, J=7Hz), 3.3-3.75(8H, m), 3.76(3H, s),

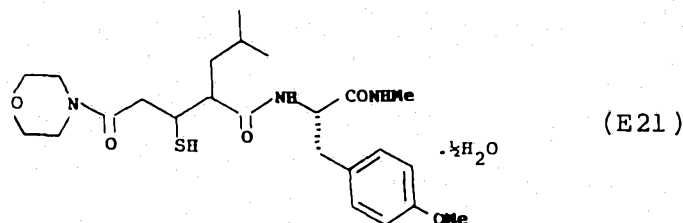
3.96(1H, q, J=6Hz), 4.54(1H, q, J=6Hz), 5.69(1H, brd),

6.61(1H, d, J=7Hz), 6.84(2H, d, J=8Hz) and

7.16(2H, d, J=8Hz).

Example 21

1-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoyl]morpholine hemihydrate (E21)



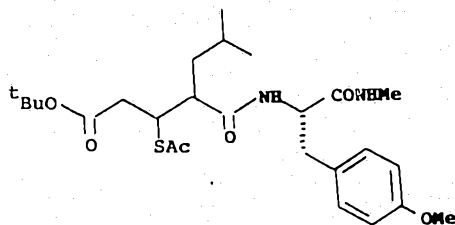
A solution of 1-[3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]-amino]carbonyl]heptanoyl]morpholine (E20, Isomer A; 12 mg) in methanol (3 ml) was purged with nitrogen and cooled in ice. The solution was treated with 35% aqueous ammonia (1.5 ml) at 0°C for 0.5 h and then allowed to warm up to room temperature. After 1.5 h at room temperature the solution was evaporated to dryness in vacuo and the residue was washed twice with ether to give the title compound as a single isomer (Isomer A) m.p. 106-108°C.

(Found: C, 58.81; H, 7.92; N, 8.75. $C_{24}H_{37}N_3O_5S \cdot 0.5H_2O$ requires C, 58.99; H, 7.83; N, 8.60%).

δ (CDCl₃): 0.87 (6H, d, J=7Hz), 1.35-1.5 (2H, m), 1.84 (1H, d, J=9Hz), 2.40 (1H, dd, J=8Hz), 2.54 (1H, m), 2.57 (1H, dd, J=4, 16Hz), 2.72 (3H, d, J=6Hz), 3.01 ((2H, d, J=7Hz), 3.32 (1H, m), 3.35-3.75 (8H, m), 3.78 (3H, s), 4.58 (1H, q, J=8Hz), 5.77 (1H, bd), 6.58 (1H, d, J=8Hz), 6.82 (2H, d, J=8Hz) and 7.15 (2H, d, J=8Hz).

Example 22

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
heptanoic acid, tert-butyl ester (E22)



(E22)

A solution of 6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
hept-2(and 3)-enoic acid, tert-butyl ester (D14; 3g) in
thiolacetic acid (15 ml) was left at room temperature
for 14 days, then evaporated to dryness in vacuo.
Column chromatography on silica gel, eluting with ethyl
acetate-pentane (1:1) gave the title compound (1.2 g)
as a mixture of isomers.

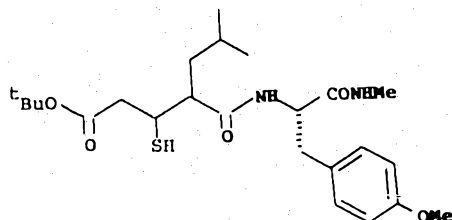
A single diastereoisomer (Isomer A), m.p. 147-151°C
(from ether-pentane), was obtained by preparative HPLC
(Lichrosorb diol), eluting with 15/85 (1% methanol/
dichloromethane)/hexane.

(Found: C, 61.08; H, 7.94; N, 5.53. C₂₆H₄₄N₂O₆S requires
C, 61.39; H, 7.93; N, 5.51%).

δ (CDCl₃): 0.84(d, H=5Hz) and 0.86(d, J=5Hz) (total 6H),
1.2-1.7(2H, m), 1.44(9H, s), 2.30(3H, s),
2.49(1H, dd, J=7, 17Hz), 2.58(1H, dd, J=5, 17Hz), 2.60(1H, m),
2.70(3H, d, J=5Hz), 2.93(1H, dd, J=8, 14Hz),
3.04(1H, dd, J=7, 14Hz), 3.79(3H, s), 3.92(1H, q, J=6Hz),
4.52(1H, q, J=7Hz), 5.52(1H, brs), 6.37(1H, d, J=8Hz),
6.82(2H, d, J=8Hz) and 7.15(2H, d, J=8Hz).

Example 23

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid, tert-butyl ester (E23)



(E23)

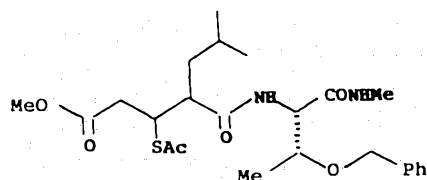
An ice-cooled solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid, tert-butyl ester (E22) (23 mg) in nitrogen-purged methanol (3 ml) was treated with 35% aqueous ammonia (1.5 ml) and the reaction mixture was stirred under nitrogen for 3 h. The solution was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was partitioned between chloroform (5 ml) and water (2 ml). The organic layer was dried (MgSO₄) and evaporated to dryness in vacuo. The product was recrystallised from ether-hexane to give the title compound, (Isomer A), m.p. 133-137°C.

(Found: C, 61.72; H, 8.45; N, 5.90. C₂₄H₃₈N₂O₅S requires C, 61.77; H, 8.21; N, 6.00%).

δ (CDCl₃): 0.86 (6H, d, J=5Hz), 1.3-1.75 (2H, m), 1.46 (9H, s), 1.65 (1H, d, J=9Hz), 2.25 (1H, dd, J=8, 14Hz), 2.44 (1H, m), 2.55 (1H, dd, J=4, 14Hz), 2.72 (3H, d, J=5Hz), 3.02 (2H, d, J=7Hz), 3.20 (1H, m), 3.77 (3H, s), 4.59 (1H, q, J=7Hz), 5.77 (1H, brd), 6.44 (1H, d, J=8Hz), 6.82 (2H, d, J=8Hz) and 7.15 (2H, d, J=8Hz).

Example 24

3-Acetylmercapto-6-methyl-4-[[[2-(R)-benzyloxy-1-(S)-[(methylamino)carbonyl]propyl]amino]carbonyl]-heptanoic acid, methyl ester (E24)



(E24)

A solution of 6-methyl-4-[[[2-(R)-benzyloxy-1-(S)-[(methylamino)carbonyl]propyl]amino]carbonyl]hept-2(and 3)-enoic acid, methyl esters (D15; 4.4 g) in thiolacetic acid (25 ml) was left at room temperature for 21 days, then was evaporated to dryness in vacuo and chromatographed on silica gel (150 g). Elution with ethyl acetate/pentane (1:1) gave the title compound as a 3:2 mixture of diastereoisomers (1.7 g) (Isomers A/B), m.p. 105-106°C (from ether-pentane). (Found: C, 59.95; H, 7.74; N, 5.84. C₂₄H₃₆N₂O₆S requires C, 59.98; H, 7.55; N, 5.83%).

δ (CDCl₃): 0.90(m), 1.11(d, J=5Hz) and 1.15(d, J=5Hz) (ratio 3:2), 1.33(m), 1.53(m), 1.68(m) 2.32(s), 2.6-2.85(m), 3.66(s) and 3.67(s) (ratio 3:2), 4.08(m), 4.55(brs), 4.67(s), 6.45(brs), 6.75(d, J=6Hz), 6.84(d, J=6Hz) and 7.35(m).

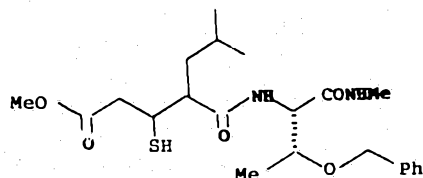


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Further elution gave the title compound as a 2:1 mixture of diastereoisomers (2.1 g) (Isomers C/D), m.p. 111-113°C (from ether-pentane).
(Found: C, 60.35; H, 7.25; N, 5.84. $C_{24}H_{36}N_2O_6S$ requires C, 59.98; H, 7.55; N, 5.83%).
 δ ($CDCl_3$): 0.88(m), 1.13(t, J=5Hz), 1.32(m), 1.52(m), 1.62-1.80(m), 2.33(s), 2.6-2.82(m), 3.64(s), 3.66(s), 4.0-4.5(m), 4.19-4.24(m), 4.50(m), 4.58-4.64(m), 6.62(m), 6.79(m) and 7.3(m).

Example 25

3-Mercapto-6-methyl-4-[[[2-(R)-benzyloxy-1-(S)-
[(methylamino)carbonyl]propyl]amino]carbonyl]heptanoic
acid, methyl ester (E23)



(E25)

Each of the mixtures of diastereoisomers (A/B and C/D) of the S-acetyl compound of Example 24 were individually treated with 35% aqueous ammonia in the usual manner, to give the title compound;

Isomer (A/B) (3:2 ratio of diastereoisomers), m.p. 113-119°C (from H₂O). (Found: C, 59.04; H, 7.88; N, 6.26. C₂₂H₃₄N₂O₅S 0.5H₂O requires C, 57.87; H, 7.95; N, 6.13%).

δ (CDCl₃): 0.94(m), 1.12(d, J=5Hz), 1.13(d, J=5Hz), 1.38(m), 1.5(m), 1.71(mm), 1.90(0.6H, d, J=8Hz), 2.06(0.4H, d, J=8Hz), 2.57(m), 2.82(m), 3.37(m), 3.72(s), 4.14(m), 4.59(brs), 4.67(s), 6.5(m), 6.78(m) and 7.33(m).

- 75 -

Isomer (C/D) (2:1 ratio of diastereoisomers), m.p.

72-76°C (from ether). (Found: C, 60.33; H, 7.97;

N, 6.40. $C_{22}H_{34}N_2O_5S$ requires C, 60.25; H, 7.81;

N, 6.39%).

δ ($CDCl_3$): 0.95(m), 1.15(t, $J=6$ Hz), 1.3(m), 1.41(m),

1.75(m), 2.01(0.66H, d, $J=8$ Hz), 2.21(0.33H, d, $J=8$ H),

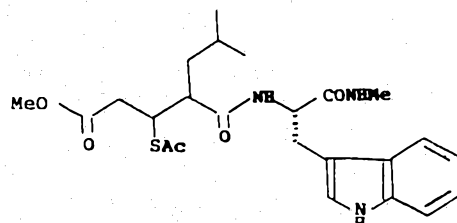
2.54(m), 2.66(m), 2.82(m), 3.37(m), 3.72(s), 4.2(m),

4.38(m)), 4.6(m), 6.55(br. d, $J=8$ Hz) 6.7(m), 6.9(br. s)

and 7.35(m).

Example 26

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]heptanoic
acid, methyl ester (E26)

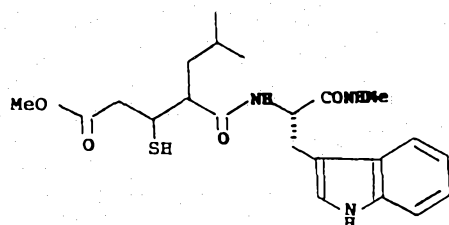


(E26)

A solution of 6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]hept-2 (and
3)-enoic acid, methyl ester (D16; 2.5 g) in thiolacetic
acid (100 ml) was left at room temperature for 28 days,
then was evaporated to dryness in vacuo. Column
chromatography (150 g) of the residue eluting initially
with ether, and then ether-chloroform (1:1) gave the
title compound (0.32 g) as a single isomer (Isomer A),
m.p. 134-136°C (from ether). (Found: C, 60.32; H, 6.88;
N, 8.80. C₂₄H₃₃N₃O₅S requires C, 60.61; H, 6.99;
N, 8.83%).
δ (CDCl₃): 0.84(d, J=5Hz) and 0.85(d, J=5Hz) (total 6H),
1.2-1.75 (2H, m), 2.29(3H, s), 2.5-2.7(3H, m),
2.64(3H, d, J=5Hz), 3.11(1H, dd, J=8, 14Hz),
3.3(1H, dd, J=6, 14Hz), 3.62(3H, s), 3.94(1H, m),
4.72(1H, q, J=7Hz), 5.64(1H, brd), 6.52(1H, d, J=8Hz),
7.05-7.25(3H, m), 7.36(1H, d, J=7Hz), 7.72(1H, d, J=7Hz) and
8.17(1H, s).

Example 27

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]heptanoic acid, methyl ester (E27)

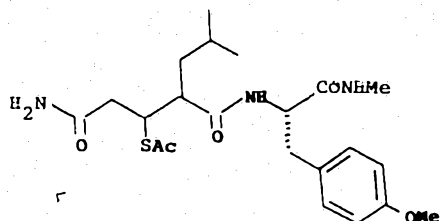


(E27)

An ice-cooled solution of 3-acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-2-(3-indolyl)ethyl]-amino]carbonyl]heptanoic acid, methyl ester (E26; 0.1 g) in nitrogen-purged methanol (5 ml) was treated with 35% aqueous ammonia (1.5 ml). The mixture was stirred at room temperature under nitrogen for 2 h. The solution was evaporated to dryness in vacuo and the residue was triturated with cold ether to give the title compound as a single isomer (Isomer A), (52 mg), m.p. 73-75°C. (Found: C, 60.69; H, 7.35; N, 9.64%. $C_{22}H_{31}N_3O_4S$ requires C, 60.95; H, 7.21; N, 9.69%). δ (CDCl₃): 0.87 (6H, d, J=6Hz), 1.4-1.7 (2H, m), 1.67 (1H, d, J=9Hz), 2.37 (1H, dd, J=5, 15Hz), 2.41 (1H, m), 2.60 (1H, dd, J=3, 15Hz), 2.65 (3H, d, J=5Hz), 3.13 (1H, dd, J=7, 14Hz), 3.21 (1H, m), 3.30 (1H, dd, J=7, 14Hz), 3.67 (3H, s), 4.75 (1H, q, J=7Hz), 5.68 (1H, brd), 6.53 (1H, d, J=8Hz), 7.05-7.25 (3H, m), 7.35 (1H, d, J=8Hz), 7.71 (1H, d, J=8Hz) and 8.13 (1H, s).

Example 28

3-Acetylmercapto-N'-[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)-pentanediamide (E28)

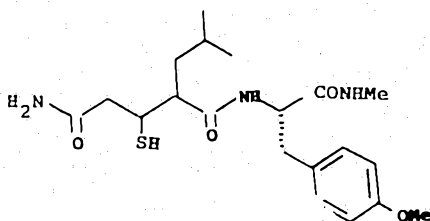


(E28)

The title compound, m.p. 235-240°C, was prepared from the compound of Example 5 and ammonia by an analogous procedure to that described in Example 11.

Example 29

3-Mercapto-N'-[1-(S)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)-pentanediamide (E29)

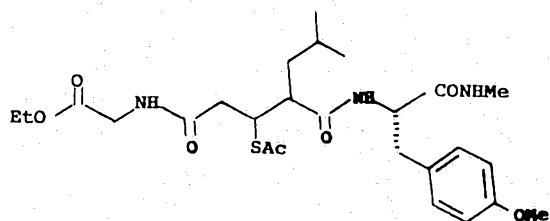


(E29)

The title compound, m.p. 221-225°C, was prepared from the compound of Example 28 by an analogous procedure to that described in Example 10.

Example 30.

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]-
carbonyl]heptanoylglycine, ethyl ester (E30)

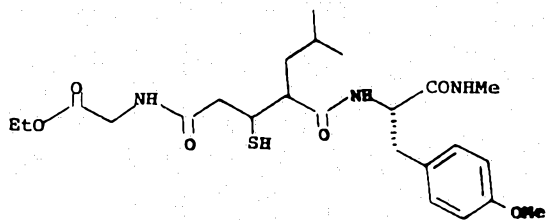


(E30)

The title compound, m.p. 190-198°C, was prepared from the compound of Example 5 and ethylglycine hydrochloride by an analogous procedure to that described in Example 12.

Example 31.

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]-
carbonyl]heptanoylglycine ethyl ester (E31)

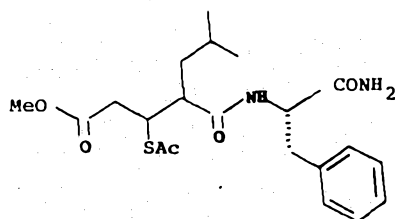


(E31)

The title compound, m.p. 221-228°C, was prepared from the compound of Example 30 by an analogous procedure to that described in Example 10.

Example 32

3-Acetylmercapto-6-methyl-4-[[[1-(S)-(aminocarbonyl)-2-phenylethyl]amino]carbonyl]heptanoic acid, methyl ester (E32)

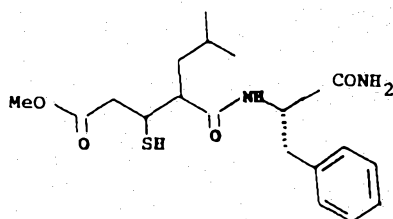


(E32)

The title compound was prepared from 6-methyl-4-[[[1-(aminocarbonyl)-2-phenylethyl]amino]carbonyl]-hept-2(and 3)-enoic acid, methyl ester and thiolacetic acid by an analogous procedure to that described in Example 26. Silica gel chromatography gave mixtures of diastereoisomers (Isomer A/B), m.p. 160-164°C and (Isomer C/D), m.p. 50-54°C (after trituration with ether). The intermediate hept-2(and 3)-enoic acids were prepared from 4-methoxycarbonyl-2-(2-methylpropyl)but-2-enoic acid and L-phenylalanine as described in Description 12.

Example 33

3-Mercapto-6-methyl-4-[[[1-(S)-(aminocarbonyl)-2-phenylethyl]amino]carbonyl]heptanoic acid, methyl ester (E33)

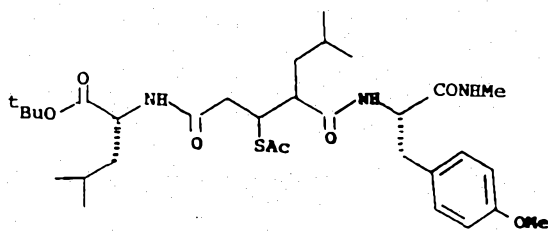


(E33)

The title compound, isolated as a mixture of diastereoisomers, (Isomer A/B), and (Isomer C/D), m.p. 64-66°C was prepared from the compound of Example 32 by an analogous procedure to that described in Example 10. The crude product was purified by silica gel chromatography using ethyl acetate as the eluent.

Example 34

3-Acetylmercapto-6-methyl-4-[[[1-(S)-
[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoyl-D-leucine, t-butyl ester (E34)

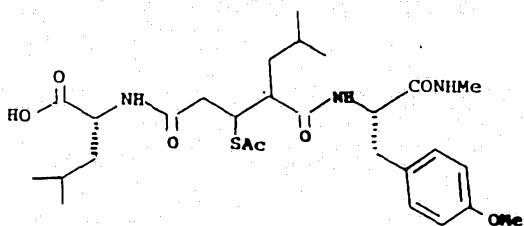


(E34)

The title compound was prepared from the compound of Example 5 and t-butylleucine hydrochloride by an analogous procedure to that described in Example 12. The product melted at 148-157°C.

Example 35

3-Acetylmercapto-6-methyl-4-[[[1-(S)-
[(Methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoyl-D-leucine, (E35)

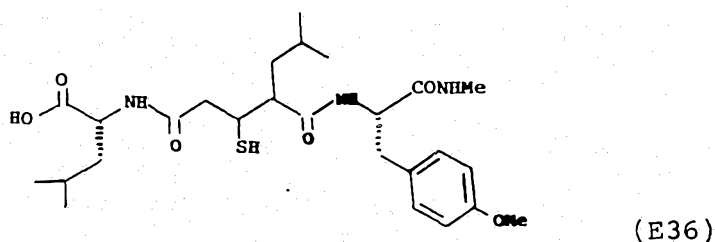


(E35)

The title compound was prepared from the compound of Example 34 using an analogous procedure to that described in Example 14. The product melted at 195°-200°C.

Example 36

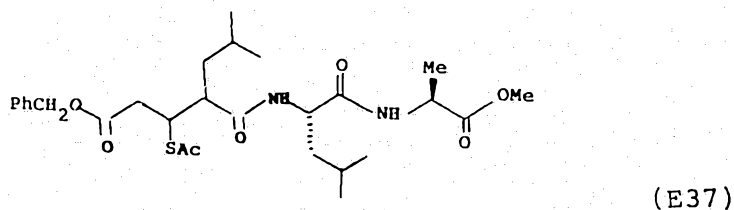
3-Mercapto-6-methyl-4-[[[1-(S)-
[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoyl-D-leucine (E36)



The title compound was prepared from the compound of Example 35 using an analogous procedure to that described in Example 15. The product melted at 216°-220°C.

Example 37

N-[N-[3-Acetylmercapto-2-(2-methylpropyl)-1-oxo-4-
[(phenylmethoxy)carbonyl]butyl]]-L-leucyl-L-alanine,
methyl ester. (E37)

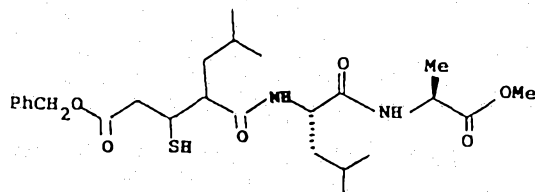


The title compound was prepared from the compound of Description 7 by an analogous procedure to that described in Example 32 using L-leucyl-L-alanine, methyl ester. The title compound was isolated as a hygroscopic foam.



Example 38

N-[N-[3-Mercapto-2-(2-methylpropyl)-1-oxo-4-
[(phenylmethoxy)carbonyl]butyl]]-L-leucyl-L-alanine,
methyl ester. (E38)



(E38)

The title compound was prepared from the compound of Example 37 by an analogous procedure to that described in Example 10. The title compound was isolated as a mixture of diastereoisomers, (Isomer A/B, 1:1), mp. 106-110° C.



COLLAGENASE INHIBITOR ASSAY

The test is performed essentially as in Cawston and Barrett Anal. Biochem. 99, 340-345 (1979). Compounds for testing are dissolved in methanol and added to purified rabbit bone collagenase in buffer. After a 5-15 min incubation at 37°C, the assay tubes are cooled to 4°C and ¹⁴C-acetylated rat skin Type I collagen is added. The assay tubes are incubated at 37°C overnight. The ¹⁴C-collagen forms insoluble fibrils which are the substrate for the enzyme.

To terminate the assay, the assay tubes are spun at 12000 rpm for 15 min. Undigested ¹⁴C-collagen is pelleted, digested ¹⁴C-collagen is found as soluble peptides in the supernatant. A sample of the supernatant is taken for liquid scintillation counting.

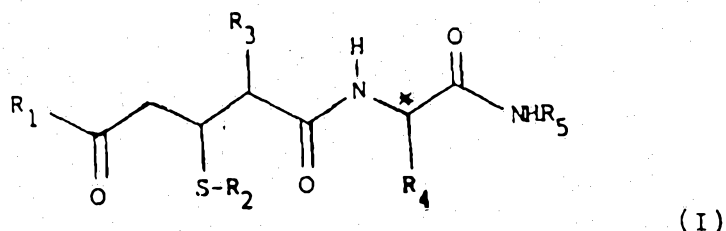
The activity of collagenase inhibitors is expressed as that amount of compound which inhibits a known amount of enzyme by 50% (IC₅₀).

The activities of representative compounds of the invention, in the above test procedure, are illustrated in the table below:

Example No.	Isomer	IC ₅₀ (nM)
2	mixture of diastereoisomers	100
2	A	3.9-6.4 (2 expts).
2	B	72
4	A	31
4	B	270
4	C	230
4	D	12600
8	A	4.7-14 (3 expts.)
10	A	8.3

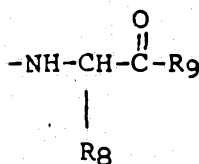
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A compound of the formula (I) or a salt, solvate or hydrate thereof:



in which:

R_1 is -OH; alkoxy; aryloxy; aralkyloxy; $-NR_6R_7$ where each of R_6 and R_7 is hydrogen or alkyl; or R_6 and R_7 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen, sulphur or optionally substituted nitrogen atom in the ring; or R_1 is a group



where R_8 is hydrogen; alkyl optionally substituted by -OH, alkoxy, $-NR_6R_7$ (wherein R_6 and R_7 are as defined above), guanidine, $-CO_2H$, $CONH_2$, -SH or -S-alkyl; or $-CH_2-Ar$ where Ar is optionally substituted aryl; and R_9 is alkoxy; OH; or $-NR_6R_7$ (wherein R_6 and R_7 are as defined above);

R_2 is hydrogen; $-C(=O)-alkyl$; or $-C(=O)-Z$, where Z is optionally substituted aryl;

R_3 is C_{3-6} alkyl;



-87-

R₄ is hydrogen; alkyl; -CH₂-R₁₀ where R₁₀ is optionally substituted phenyl or heteroaryl; or a group

-CH-O-R₁₁

|
R₁₂

where R₁₁ is hydrogen; alkyl; or -CH₂-Ph where Ph is optionally substituted phenyl; and R₁₂ is hydrogen or alkyl; and

R₅ is hydrogen or alkyl; or a group

-CH COR₁₄

|
R₁₃

where R₁₃ is hydrogen; or alkyl; and R₁₄ is hydroxy; alkoxy; or -NR₆R₇ (wherein R₆ and R₇ are as defined above).

2. A compound according to claim 1 in which R₁ is hydroxy, C₁₋₄ alkoxy, benzyloxy, amino, methylamino, N¹-methyl-N-piperazinyl, N-morpholinyl or -NH-CH(R₈)COR₉ where R₈ is hydrogen or iso-butyl and R₉ is OH, NH₂, ethoxy or t-butoxy;

R₂ is hydrogen, acetyl, or benzoyl;

R₃ is n-butyl, iso-butyl or sec-butyl;

R₄ is iso-butyl, benzyl, 4-methoxy benzyl, 1-(benzyloxy)ethyl or 3-indolylmethyl; and

R₅ is hydrogen, methyl, or 1-(methoxycarbonyl)ethyl.



3. A compound according to claim 1 in which R₁ is methoxy, amino, methylamino or -NHCH₂CO₂H; R₂ is hydrogen, acetyl or benzyl; R₃ is ^{or 3-indolyl, acetyl,} iso-butyl; R₄ is benzyl, 4-methoxybenzyl and R₅ is methyl.

4. A compound according to any one of claims 1 to 3 in which the chiral centre marked with an asterisk in formula (1) has the S configuration.

5. A compound according to claim 1 which is:

3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-heptanoic acid, methyl ester,

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-heptanoic acid, methyl ester,

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-heptanoic acid,

3-Mercapto-N⁵-methyl-N'[1-(S)-[methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)-pentanediamide,

2-[3-Mercapto-6-methyl-4[[[1-(S)-[(methylamino)-carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-heptanoylamino]ethanoic acid,



01
02
03
04
05
06 N⁵-(Aminocarbonyl)methyl-3-mercapto-N'-[1-(S)-
07 [(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-
08 (2-methylpropyl)pentanediamide,
09
10

11
12
13
14 3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
15 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
16 heptanoic acid, benzyl ester,
17

18 3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
19 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
20 heptanoic acid, benzyl ester,
21

22 3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
23 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
24 heptanoic acid,
25

26 3-Benzoylmercapto-N⁵-methyl-N¹-[1-(S)-[(methylamino)-
27 carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)-
28 pentanediamide,
29

30 3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
31 carbonyl]-2-phenylethyl]amino]carbonyl]heptanoic acid,
32 methyl ester,
33

34 3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
35 carbonyl]-2-phenylethyl]amino]carbonyl]heptanoic acid,
36 methyl ester,
37

01 3-Acetylmercapto-N⁵-methyl-N¹-[1-(S)-
02 [(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-
03 (2-methylpropyl)pentanediamide,
04

05
06 2-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
07 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-
08 anoylamino]ethanoic acid, t-butyl ester,
09

10 2-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
11 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
12 heptanoylamino]ethanoic acid, t-butyl ester,
13

14 2-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-
15 [(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
16 amino]carbonyl]heptanoylamino]ethanoic acid,
17

18 3-Acetylmercapto-N⁵-(aminocarbonyl)methyl-N¹-[1-(S)-
19 [(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-
20 methylpropyl)pentanediamide,
21

22 1-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
23 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
24 heptanoyl]-4-methylpiperazine,
25

26 1-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
27 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
28 heptanoyl]-4-methylpiperazine,
29

30 1-[3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
31 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
32 heptanoyl]morpholine hydrate,
33

34 1-[3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
35 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
36 heptanoyl]morpholine hemihydrate,
37



01
02 3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
03 carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]-
04 heptanoic acid, tert-butyl ester,

05
06 3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-
07 2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic acid,
08 tert-butyl ester,

09
10 3-Acetylmercapto-6-methyl-4-[[[2-(R)-benzyloxy-1-
11 (S)-[(methylamino)carbonyl]propyl]amino]carbonyl]-
12 heptanoic acid, methyl ester,

13
14 3-Mercapto-6-methyl-4-[[[2-(R)-benzyloxy-1-(S)-
15 [(methylamino)carbonyl]propyl]amino]carbonyl]heptanoic
16 acid, methyl ester,

17
18 3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
19 carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]heptanoic
20 acid, methyl ester,

21
22 3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)carbonyl]-
23 2-(3-indolyl)ethyl]amino]carbonyl]heptanoic acid,
24 methyl ester,

25
26 3-Acetylmercapto-N'-[1-(S)-[(methylamino)carbonyl]-2-
27 (4-methoxyphenyl)ethyl]-2-(2-methylpropyl)-
28 pentanediamide,

29
30 3-Mercapto-N'-[1-(S)-[(methylamino)carbonyl]-2-
31 (4-methoxyphenyl)ethyl]-2-(2-methylpropyl)
32 pentanediamide,

33
34 3-Acetylmercapto-6-methyl-4-[[[1-(S)-[(methylamino)carb -
35 onyl]-2-(4-methoxyphenyl)ethyl]amino]-
36 carbonyl]heptanoylglycine, ethyl ester,

37



3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]amino]-
carbonyl]heptanoylglycine, ethyl ester,

3-Acetylmercapto-6-methyl-4-[[[1-(S)-
(aminocarbonyl)-2-phenylethyl]amino]carbonyl]heptanoic
acid, methyl ester,

3-Mercapto-6-methyl-4-[[[1-(S)-(aminocarbonyl)-
2-phenylethyl]amino]carbonyl]heptanoic acid, methyl
ester,

3-Acetylmercapto-6-methyl-4-[[[1-(S)-
[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoyl-D-leucine, t-butyl ester,

3-Acetylmercapto-6-methyl-4-[[[1-(S)-
[(Methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoyl-D-leucine,

3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(4-methoxyphenyl)ethyl]-
amino]carbonyl]heptanoyl-D-leucine,

N-[N-[3-Acetylmercapto-2-(2-methylpropyl)-1-oxo-4-
[(phenylmethoxy)carbonyl]butyl]]-L-leucyl-L-alanine
methyl ester, or

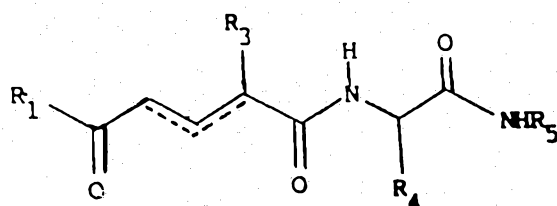
N-[N-[3-Mercapto-2-(2-methylpropyl)-1-oxo-4-
[(phenylmethoxy)carbonyl]butyl]]-L-leucyl-L-alanine
methyl ester.

6. 3-Mercapto-6-methyl-4-[[[1-(S)-[(methylamino)-
carbonyl]-2-(3-indolyl)-ethyl]amino]carbonyl]heptanoic
acid, methyl ester.

7. A process for the preparation of a compound of
formula (I) as claimed in claim 1, which process
comprises:

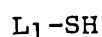


(a) the reaction of a compound of formula (III):



(III)

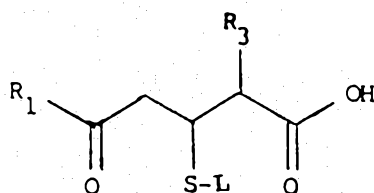
in which R_1 , R_3 , R_4 and R_5 are as defined in formula (I) with a thiol of formula (IV):



(IV)

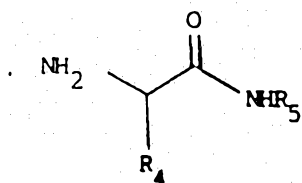
in which L_1 is a conventional sulphur protecting group;
or

(b) the reaction of a compound of formula (V):



(V)

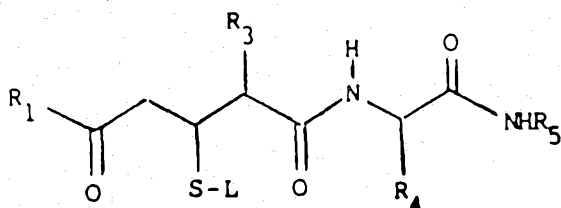
in which R_1 and R_3 are as defined in formula (I) and L is L_1 which is a conventional sulphur protecting group, or L_2 which is a group $R-S-$ where R is any organic residue such that the group $R-S-$ provides a cleavable disulphide bond, with a compound of formula (VI):



(VI)



in which R_4 and R_5 are as defined in formula (I), to give as the product of process variant (a) or (b) a compound of formula (II):



(II)

in which R_1 , R_3 , R_4 and R_5 are as defined in formula (I) and L is L_1 or L_2 ; and subsequently, where R_2 in a compound of formula (I) is hydrogen, or L is other than acyl cleaving the group L.

8. A process according to claim 7 in which L is L_1 where L_1 is acetyl or benzoyl.

9. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 6 and a pharmaceutically acceptable carrier.

10. A method of treating collagenolytic disorders, the method comprising the administration of a compound of formula (I) as defined in any one of claims 1 to 6 to a mammal in need thereof.

11. Compounds of formula (I) or salts, solvates or hydrates thereof, methods for their manufacture or pharmaceutical compositions or methods of treatment involving them, substantially as hereinbefore described with reference to the Examples.

DATED this 14th day of May 1990.

BEECHAM GROUP p.l.c.,
By Its Patent Attorneys
DAVIES & COLLISON

