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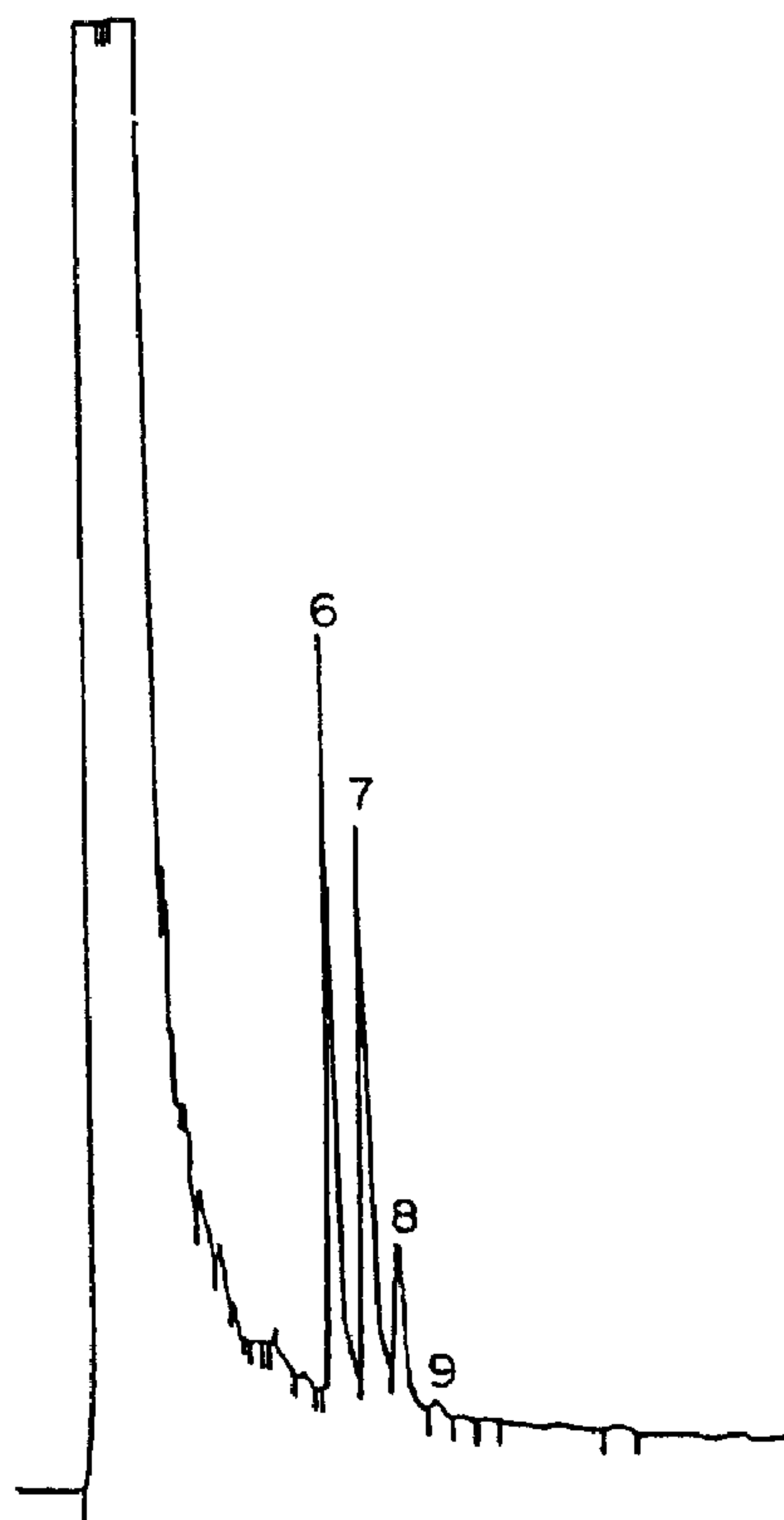
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(54) **STABILISANTS DE MOUSSE ET AMERISANTS DERIVES DES
CONES DE HOUBLON**

(54) **HOPS-DERIVED FOAM STABILITY AND BITTERING AGENTS
ISOMERIZED- α -ACIDS**



(57) On utilise comme agents stabilisants la mousse et lui donnant son amertume, des acides dihydro-iso-alpha dérivés du houblon, plus particulièrement de la dihydro-iso-humulone et de la dihydro-iso-adhumulone, et de "l'iso-adpréhumulone".

(57) Hops-derived dihydro-iso-alpha-acids, in particular dihydro-iso-humulone and dihydro-iso-adhumulone, and "iso-adpre-humulone" are employed as foam stabilizing and bittering agents.



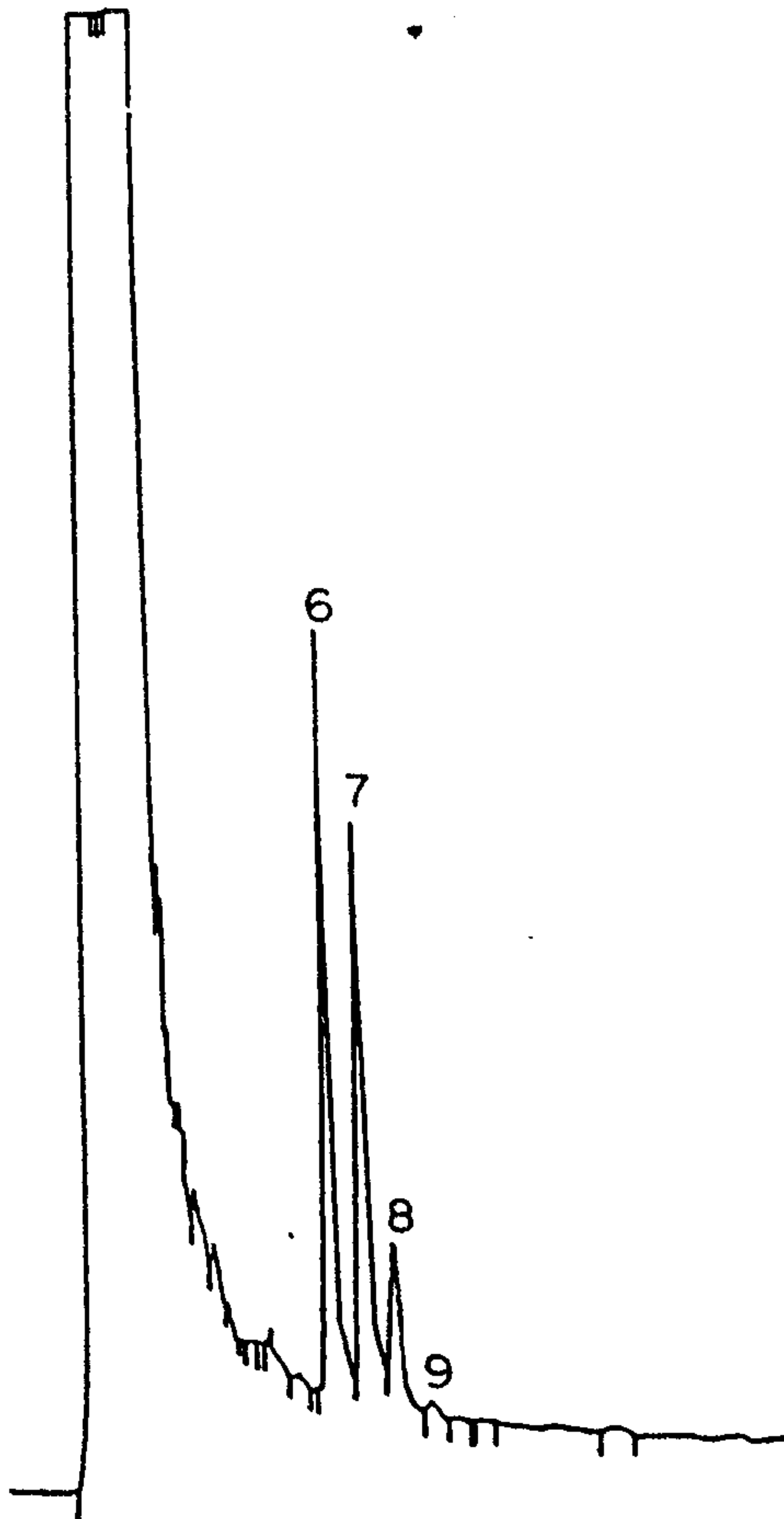


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(21) International Application Number: PCT/US92/05754 (22) International Filing Date: 9 July 1992 (09.07.92) (30) Priority data: 729,922 15 July 1991 (15.07.91) US (60) Parent Application or Grant (63) Related by Continuation US 729,922 (CIP) Filed on 15 July 1991 (15.07.91) (71) Applicant (for all designated States except US): S.S. STEIN- ER, INC. [US/US]; 655 Madison Avenue, New York, NY 10021-8078 (US).			(72) Inventors; and (75) Inventors/Applicants (for US only) : SMITH, Robert, J. [US/US]; 300 S. 76th Avenue, No. 30, Yakima, WA 98908 (US). WILSON, Richard, J., H. [GB/GB]; 29 Newlands Park, Copthorne, West Sussex RH10 3EW (GB). (74) Agents: SOLOWAY, Norman, P. et al.; Hayes, Soloway, Hennessey & Hage, 175 Canal Street, Manchester, NH 03101-2335 (US). (81) Designated States: AU, CA, CS, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>

(54) Title: HOPS-DERIVED FOAM STABILIZING AND BITTERING AGENTS**(57) Abstract**

Hops-derived dihydro-iso-alpha-acids, in particular dihydro-iso-humulone and dihydro-iso-adhumulone, and "iso-adprehumulone" are employed as foam stabilizing and bittering agents.



1 HOPS-DERIVED FOAM STABILITY AND BITTERING
2 AGENTS ISOMERIZED- α -ACIDS

3

4 Field of the Invention

5 This invention relates to the production of hops
6 derived compounds suitable for bittering beer, and for
7 improving the foam stability of beer.

8 Background of the Invention

9 In the traditional process of brewing beer, hops are
10 boiled with wort to extract the bittering principles and
11 other flavor components. The wort is then cooled and
12 fermented by addition of yeast. After fermentation, the
13 yeast is removed and a clarified beer produced. It is now
14 known that the "natural" beer bitterness produced in a
15 traditional brewing process is largely derived from a
16 group of compounds known as alpha-acids, that are present
17 in the resin fraction of the hop lupulin glands. These
18 compounds are extracted into the boiling wort and then may
19 undergo chemical reaction to form a variety of substances,
20 chief of which are the iso-alpha-acids. It is these iso-
21 alpha-acids that are largely responsible for the bitter
22 flavor in beer. Another important function of the iso-
23 alpha-acids is to assist in the formation of the foam head
24 that forms when carbonated beer is poured into a glass.
25 Beer foam has a complex structure, but is primarily
26 dependent on the presence of certain proteins that have
27 both hydrophobic and hydrophilic regions. The presence of
28 iso-alpha-acids is well known to stabilize beer foam by
29 interacting in a positive fashion with the proteins and
30 encouraging the formation of a head that has a greater
31 density of gas bubbles per unit volume. Such foam usually
32 shows an increased propensity to exhibit enhanced "lacing"
33 and "cling" on the sides of the glass as the beer is

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1 drunk, characteristics that are generally perceived as
2 aesthetically pleasing to the consumer.

3 It has long been known that the so-called
4 "utilization" of hop alpha-acids added to the wort kettle
5 in traditional brewing practice is poor. Typically, only
6 30-40% of the added alpha-acids appear subsequently as
7 iso-alpha-acids in the beer. For example, if the hops are
8 insufficiently boiled in the wort, then much of the alpha-
9 acids may remain unconverted. Another source of
10 inefficiency are the many side reactions of alpha-acids
11 that occur during wort boiling, producing compounds that
12 are not necessarily bitter. Also, the boiling of wort
13 inevitably produces a proteinaceous precipitate referred
14 to as "trub" or "hot break" and it is known that this
15 material tends to attract deposition of iso-alpha-acids.
16 Normal brewery practice is to remove most of this material
17 by filtration or, more commonly, by the action of a
18 whirlpool separator. About 20% of the iso-alpha-acids
19 present in the whole wort may be lost in this way.
20 Subsequent cooling of the hot wort leads to formation of
21 further precipitated organic material (the "cold break")
22 which again may absorb a proportion of the iso-alpha-
23 acids. Further losses of dissolved iso-alpha-acids occur
24 during the fermentation of the wort. Adsorption onto the
25 virgin walls of multiplying yeast cells occurs, no doubt
26 encouraged by the falling pH value of the wort as it is
27 transformed into beer. Furthermore, a tendency for the
28 iso-alpha-acids to concentrate in the foam head that
29 usually forms in the absence of added antifoams often
30 results in deposition and subsequent loss of iso-alpha-
31 acids on the walls of the fermenting vessel. Overall, a
32 loss of 20-40% of the iso-alpha-acids through the

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1 fermentation stage is to be expected. Finally, a further
2 significant loss may occur during fining and filtration
3 before the beer is eventually packaged.

4 In order to maximize the utilization of alpha-acids,
5 it was long ago proposed that addition of iso-alpha-acids
6 post-fermentation would be advantageous, provided that one
7 could efficiently prepare a suitable formulation of these
8 bittering substances from the natural hop alpha-acids.
9 Several processes for the preparation of so-called
10 "isomerized extracts" have been devised. For example, in
11 U.S. Patent No. 3,364,265, Klingel et al describe the
12 production of iso-alpha-acids in sodium salt form obtained
13 via hot alkaline aqueous solution treatment of alpha-acids
14 obtained from organic solvent extraction of hops. In U.S.
15 Patent No. 3,973,052, Mitchell describes the production of
16 highly concentrated aqueous solutions of potassium iso-
17 alpha-acid salts that are particularly convenient to
18 handle and may be added directly to beer. More recently,
19 Forrest, Seaton and Moir in U.S. Patent No. 4,212,895
20 demonstrate the production of such a "liquid" isomerized
21 extract from hop resin extracts prepared by extraction of
22 hops using liquid or supercritical carbon dioxide. CO₂
23 extracts are especially suitable for use as raw material
24 for production of isomerized extracts because they are
25 generally of higher purity, often being essentially free
26 from hard resins, chlorophyll and much of the hop waxes
27 which may be present in extracts commonly prepared by use
28 of organic solvents such as dichloromethane, ethanol or
29 hexane. The process of U.S. Patent No. 4,212,895 is of
30 particular interest as it allows production of isomerized
31 extracts from CO₂ hop extracts without the use of
32 undesirable solvents or unnatural chemical substances at

1 any stage. In this way, isomerized extracts can be
2 prepared that are virtually or totally free from traces of
3 compounds that might be harmful to health or to beer
4 flavor. Such extracts are in common use throughout the
5 brewing industry. They serve two main purposes: first, to
6 reduce costs by greatly improving the utilization of the
7 hop alpha-acids and, secondly, to provide a convenient
8 means whereby the brewer may regulate the bitterness of
9 the beer via a "topping-up" procedure. (In fact, these
10 benefits go hand in hand, for it is well known that
11 utilization of the alpha-acids added to the wort kettle is
12 increased if the quantity added is reduced.)

13 It is important during manufacture of isomerized
14 extract intended for addition to beer to remove as far as
15 is reasonably practicable the beta-acids which are natural
16 and major components of the hop resin fraction within the
17 lupulin glands. These beta-acids are chemically closely
18 related to the alpha-acids and are extracted together with
19 the alpha-acids by organic solvents or by liquid or
20 supercritical CO₂. Dependent primarily on hop variety,
21 the ratio of beta-acid to alpha-acid in a hop extract may
22 vary between about 0.25:1 to about 1.2:1. Beta-acids are
23 of little value in normal brewing as they do not isomerize
24 and are particularly insoluble in wort or beer, causing
25 undesirable hazes if present in added isomerized extract.
26 Because of their close chemical relationship to the alpha-
27 acids, it has occurred to many workers to attempt to
28 convert the beta-acids contained in a hop extract into
29 alpha-acids or other useful substances, thereby enhancing
30 the brewing value of the extract. In the course of such
31 work, many transformations of beta-acids were discovered,
32 leading not only to processes whereby beta-acids could be

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1 converted to alpha-acids and hence to iso-alpha-acids, but
2 also to other previously known or unknown derivatives of
3 both alpha-acids and beta-acids. These discoveries have
4 been reviewed by several authors, including, for example,
5 Verzele in J. Inst. Brew. 92 32-48 (1986). Processes have
6 been described for the production of several derivatives
7 of alpha-acids and beta-acids that have chemical or
8 physical properties that are of acknowledged value in
9 brewing. Important amongst these derivatives are those
10 that take the form of modified iso-alpha-acids in which
11 the properties of the natural iso-alpha-acids have, in
12 effect, been usefully altered through chemical reduction
13 of their molecular structures. This reduction takes place
14 within one or more of the three side chains present in all
15 iso-alpha-acids and involves the addition of up to six
16 hydrogen atoms in total per molecule.

17 In U.S. Patent No. 3,079,262 Hougen described a
18 process for the formation of tetrahydro-alpha-acids by
19 reduction of the alpha-acids present in hop extract using
20 hydrogen gas in the presence of a noble metal catalyst.
21 Hougen demonstrated that the tetrahydro-alpha-acids would
22 be subsequently transformed to tetrahydro-iso-alpha-acids
23 if the modified extract was then added to wort and boiled
24 in the usual way. Beer prepared in this way was bitter
25 and had the useful property of resisting formation of
26 unpleasant "light-struck" or "skunky" flavors when exposed
27 to sunlight, a phenomenon known to occur in normal beer
28 due to a reaction between iso-alpha-acids and naturally
29 present mercaptans. Subsequently, other workers described
30 routes to tetrahydro-iso-alpha-acids starting from beta-
31 acids. For example, Worden and Todd in U.S. Patent No.
32 3,522,975 showed the formation of tetrahydro-iso-alpha-

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1 acids by a three-step process firstly involving
2 hydrogenolysis to form 4 - deoxytetrahydro-alpha-acids,
3 followed by oxidation to tetrahydro-alpha-acids and
4 finally by isomerization in hot aqueous alkali to the
5 tetrahydro-iso-alpha-acids. The same patent also
6 describes the novel formation of hexahydro-iso-alpha-
7 acids, effected by further reduction of the tetrahydro-
8 iso-alpha-acids using sodium borohydride. It was noted
9 that not only was this compound bitter and of value to
10 prevent "light-struck" flavor formation, but it also
11 induced a particularly stable foam when added to beer, as
12 is also found to be true of the tetrahydro-iso-alpha-
13 acids. As it turns out, the various properties of tetra-
14 and hexa- hydrogenated iso-alpha-acids have, in practice,
15 been found to be of significant commercial value.
16 Tetrahydro-iso-alpha-acids preparations have been most
17 favored, not only because they are simpler to prepare but
18 because they are also perceived to be substantially more
19 bitter than either hexahydro-iso-alpha-acids or iso-alpha-
20 acids (Todd, Johnson & Worden, Tech. Quart. Master Brewers
21 Association of the Americas, 9, pp 31-35, [1971]).
22 A major drawback to the widespread use of tetra- (or
23 hexa-) hydro-iso-alpha-acids is the unnaturalness of these
24 compounds. Neither class of substance has ever been
25 reported as occurring naturally in beer produced from
26 hops, hop pellets or hop extracts added to the wort
27 kettle. Similarly, the presence of hydrogenated forms of
28 alpha-acids has never previously been observed to occur
29 naturally in hops or hop products not subjected to
30 deliberate chemical transformations. Brewers are
31 increasingly reluctant to use unnatural additives even

1 though proven safe for fear of alienating certain of their
2 customers who may object to "adulteration" of the product.

3 Objects of the Invention

4 It is an object of the present invention to provide
5 bittering and foam stabilizing systems, i.e. methods and
6 materials, which overcome disadvantages of the prior art.
7 A particular object of the present invention is to provide
8 a foam stabilizing material that is demonstrably natural.
9 The present invention is based on the discovery that
10 certain compounds which we find are naturally present in
11 quite minor (functionally insignificant) amounts in hops,
12 advantageously may be employed as beer foam stabilizing
13 and bittering substances after conversion to their
14 isomerized derivatives.

15 In particular, we have discovered that dihydro-alpha-
16 acids (which compounds heretofore were known only as
17 intermediates in the production of tetrahydro-alpha-
18 acids), especially dihydro-humulone, and an alpha-acid we
19 term "adprehumulone" (and having a structure believed to
20 be represented by Formula Drawing 3) are valuable in this
21 respect.

22 Description of the Drawings

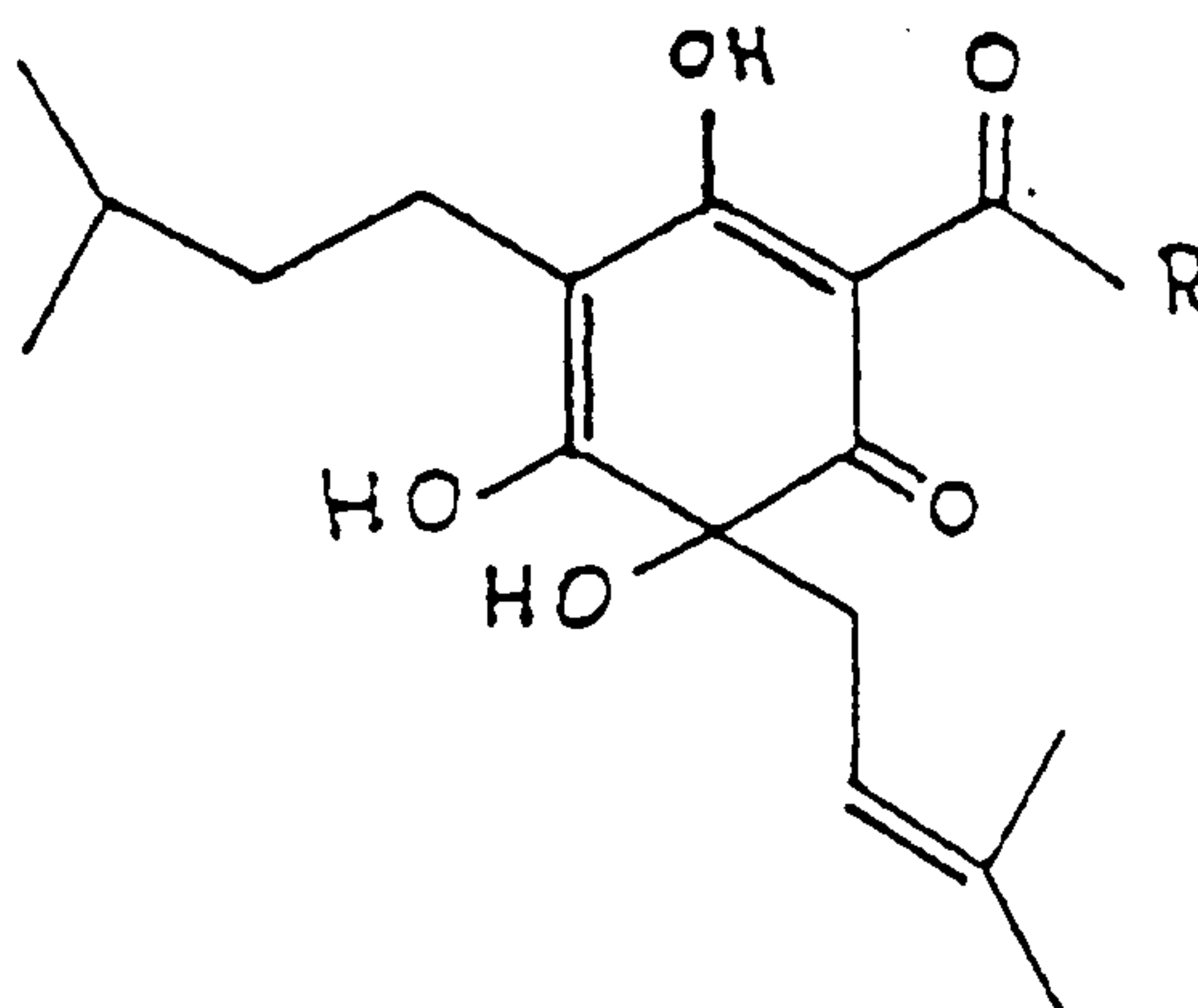
23 Further features and advantages of the present
24 invention may be seen from the following detailed
25 description of the invention taken in conjunction with the
26 drawings in which Figs. 1 to 3 are chromatograms
27 illustrating analyses of materials in accordance with the
28 present invention.

29 Detailed Description of the Invention

30 As used herein the nomenclature dihydro-alpha-acids
31 and dihydro-iso-alpha-acids and "adprehumulone" means

1 compounds of the general structures shown in Formula
2 Drawings 1-3:

3 1. Dihydro-alpha-acid



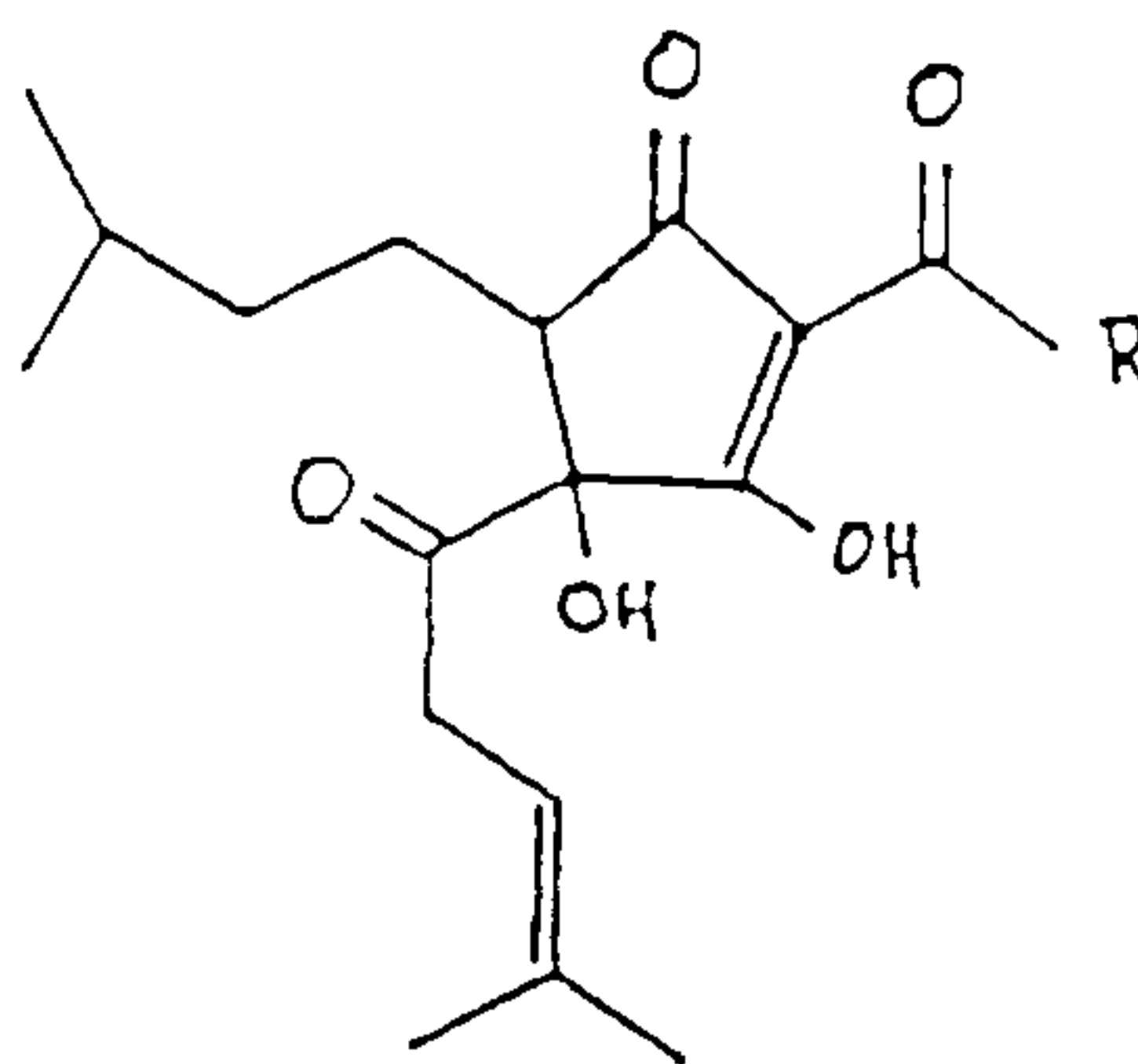
14 R is a straight or branched chain alkyl, preferably

16 -CH(CH₃)₂ (= -cohumulone)

17 -CH₂CH(CH₃)₂ (= -humulone)

18 -CH(CH₃)CH₂CH₃ (= -adhumulone)

23 2. Dihydro-iso-alpha-acid

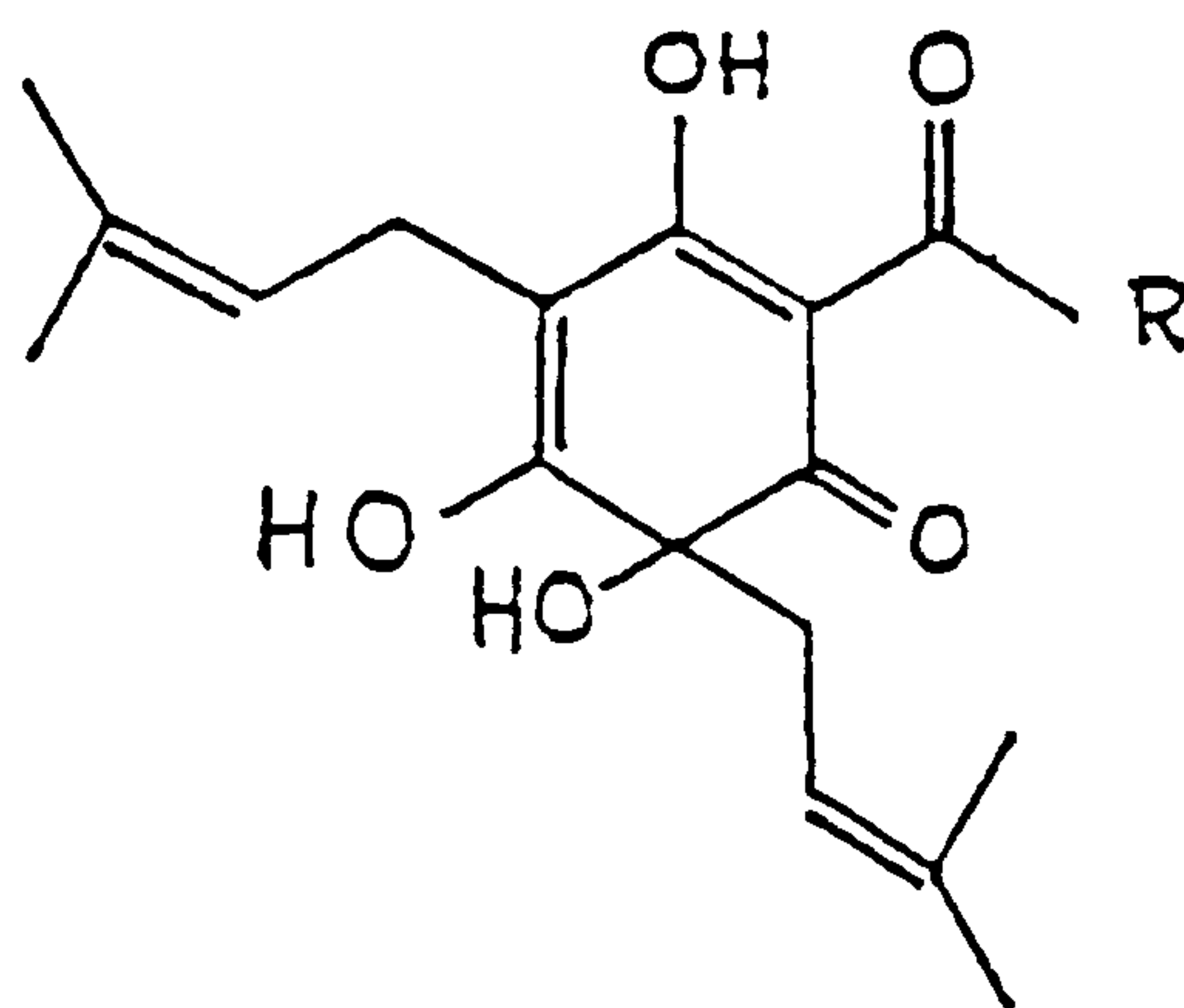


32 R groups as for Formula Drawing 1

21 13274

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3. Alpha-Acid



R

-CH₂-CH₂-CH(CH₃)₂

(=Prehumulone)

-CH₂-CH₂-CH₂-CH₂-CH₃

(="Adprehumulone")

-CH₂-CH(CH₃)-CH₂-CH₃

(=alternative
structure for
"Adprehumulone")

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1 We specifically exclude other possible dihydrogenated
2 forms of alpha-acids or iso-alpha-acids that have been
3 assigned the same nomenclature by previous authors, as for
4 example by Todd, Johnson and Worden in Tech. Quart. of the
5 Master Brewers Association of the Americas, 9 pp 31-35,
6 (1971) and by Anteunis & Verzele in Bull. Société Chimique
7 Belges, 68, 102 & 476 (1959). Dihydro-alpha-acids are
8 known to be formed as intermediates in the catalytic
9 hydrogenation of alpha-acids to tetrahydro-alpha-acids.
10 However, the use of such compounds (and in particular
11 dihydro-iso-humulone) as foam stabilizing and bittering
12 compounds has not previously been described since such
13 compounds heretofore have been considered merely to be
14 intermediates for the production of tetrahydro-iso-alpha-
15 acids. We have found that dihydro-iso-alpha-acids are
16 about as bitter as iso-alpha-acids, but are more potent
17 foam stabilizing substances than their corresponding iso-
18 alpha-acid homologs or analogs. In particular,
19 preparations containing a major proportion of isomerized
20 hop compounds as dihydro-iso-humulone have an ability to
21 enhance beer foam and cling that is similar to
22 commercially available preparations of tetrahydro-iso-
23 alpha-acids.

24 The dihydro-alpha-acids useful (after isomerization)
25 in accordance with the present invention are found to
26 exist in quite minor (functionally insignificant) amount,
27 e.g. about 0.002% by weight, in fresh, dried hops.
28 However, the concentration appears to increase with
29 mechanical processing and aging. Thus, we have observed
30 concentrations of about 0.04% by weight in one year old,
31 cold stored hop pellets; about 0.10% in three year old,
32 cold stored hop pellets; and of about 0.27% in ambient

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1 stored, three year old hop pellets. Aged extracts also
2 appear to show increased concentrations of dihydro-alpha-
3 acids. Particularly striking, a 1988 dichloromethane
4 extract (standardized with water-solubles) was found to
5 contain 3.2% of dihydro-humulone (+dihydro-adhumulone)
6 when analyzed in 1991.

7 While not wishing to be bound by theory, it is
8 believed that the formation of dihydro-alpha-acids in
9 hops, hop pellets and hop extracts is due to a hitherto
10 unknown and surprising reaction that can occur between
11 molecules of alpha-acids. This reaction may be enhanced
12 in accordance with one aspect of the present invention by
13 the addition of a sub-equivalent proportion of simple
14 alkaline substances, and results in the formation of
15 dihydro-alpha-acids and other presently unidentified
16 derivatives of alpha-acids, but without the slightest
17 detectable formation of tetrahydro- or other undesirable
18 hydrogenated derivatives of alpha-acids. This is
19 surprising since conventional catalytic hydrogenation of
20 alpha-acids to produce dihydro-alpha-acids in accordance
21 with the prior art invariably produces at least some
22 tetrahydro-alpha-acids.

23 Currently, there are two basic preferred routes by
24 which dihydro-humulone (and other dihydro-alpha-acids) may
25 be formed suitably for use as precursor to the formation
26 of dihydro-iso-humulone (and other dihydro-iso-alpha-
27 acids) in accordance with the present invention.

28 One route involves the catalytic hydrogenation of
29 alpha-acids under conditions that favor the formation of
30 dihydro-alpha-acids. The catalytic hydrogenation of
31 alpha-acids to form tetrahydro-alpha-acids is well known
32 in the prior art. Anteunis & Verzele also report in Bull.

1 Société Chimique Belges 68, pp 315-324 (1959) reaction
2 conditions under which a molar ratio yield of dihydro-
3 humulone to tetrahydro-humulone of about 4:1 can be
4 obtained. We have discovered that using certain selected
5 catalysts such as Pt/Alumina and Pt/Carbon, and by
6 conducting the hydrogenation reaction at moderately low
7 temperatures, e.g. 0° to 40°C, it is possible to achieve
8 molar ratio yields of the desired dihydro-alpha-acids to
9 tetrahydro-alpha-acids of up to about 10:1 or more. The
10 undesirable tetrahydro-alpha-acids may then be removed
11 using conventional separation techniques.

12 In the second instance, we have discovered a route to
13 the formation of dihydro-alpha-acids completely free from
14 concurrent formation of tetrahydro- or hexahydro-alpha-
15 acids. More particularly, we have discovered that alpha-
16 acids, particularly humulone, can be converted to
17 corresponding dihydro-alpha-acids in an unusual solid (or
18 near solid) state reaction in the presence of sub-
19 equivalent amounts of alkali, preferably 0.3 to 0.6
20 equivalents of alkali per mole of alpha-acids, more
21 preferably about 0.4 equivalents of alkali per mole of
22 alpha-acids, whereby the alpha-acids are partially
23 converted to their alkali metal salts. Various alkali
24 agents advantageously may be used in accordance with the
25 present invention, amongst which are mentioned NaOH, LiOH,
26 KOH, Na₂CO₃, NaHCO₃, Na₂HPO₄, K₂HPO₄ and K₂CO₃.
27 Preferably, this reaction is conducted at relatively low
28 temperature, specifically below about 80° C.

29 The alpha-acid that we term "adprehumulone" is
30 believed to be identical with one of two closely related
31 compounds tentatively identified in 1962 as present in
32 hops by Rigby, Sihto & Bars (J. Inst. Brewing (1962), 68,

1 pp 60-65), and considered at that time to be dual
2 components of an alpha-acid fraction obtainable from
3 extracted hops with the aid of countercurrent
4 distribution. This fraction was first obtained by Verzele
5 (Bull. Soc. Chim. Belg. (1955), 64, pp 70-86) and was
6 termed "prehumulone". In later work, Rillaers and Verzele
7 (in Bull. Soc. Chim. Belg. (1962), 72, pp 438-445)
8 described the purification of "prehumulone" and, with the
9 aid of NMR analysis, assigned a specific, single structure
10 to this compound that was identical to one of the two
11 prehumulones described by Rigby and co-workers. It has
12 since become commonly accepted that the term "prehumulone"
13 refers only to the compound described by Rillaers and
14 Verzele. We now demonstrate the existence of a naturally
15 occurrent alpha-acid that we tentatively identify as the
16 alternative prehumulone of Rigby and co-workers. We term
17 this substance "adprehumulone" and confirm it to be of
18 identical molecular weight but structurally
19 distinguishable from the prehumulone of Rillaers and
20 Verzele, though we do not discount the possibility that we
21 have discovered a previously unknown alpha-acid of closely
22 related structure as indicated in Formula Drawing 3. As
23 is well-known, alpha-acids, when present in beer as iso-
24 alpha-acids, are important foam stabilizers. In view of
25 the structural similarity of prehumulone and
26 "adprehumulone", it is surprising that we find iso-
27 "adprehumulone" to be a much more potent foam stabilizer
28 than iso-prehumulone.

29 Working Examples

30 The following examples include the illustration of
31 means whereby dihydro-alpha-acids may be formed either in
32 the absence or, alternatively, in the minor presence of

1 tetrahydro-alpha-acids, and the complete or virtual
2 absence of hexahydro-alpha-acids. It is to be appreciated
3 that the processes and conditions described are not
4 exclusive and therefore do not preclude other reaction
5 conditions that fall within the same basic principles.

6 Examples 1 to 3 describe the analysis of hops and hop
7 products and establish the natural presence of minor (and
8 generally functionally insignificant) amounts of dihydro-
9 alpha-acids in hops and hop products;

10 Example 4 describes the analysis of beer and
11 establishes the natural presence of minor (and
12 functionally insignificant) amounts of dihydro-iso-
13 humulone in beer;

14 Examples 5 to 10 illustrate the production of dihydro-
15 alpha-acids in functionally useful quantities in
16 accordance with the present invention;

17 Examples 11 to 13 illustrate the production of
18 dihydro-iso-alpha-acids in functionally useful quantities
19 in accordance with the present invention;

20 Example 14 illustrates the use of dihydro-iso-alpha-
21 acids as foam stabilizing agents in accordance with the
22 present invention;

23 Example 15 demonstrates the preparation of
24 hydrogenated, isomerized resin extract (IRE) in
25 functionally useful quantities in accordance with the
26 present invention;

27 Example 16 illustrates the use of hydrogenated IRE as
28 a foam stabilizing agent in accordance with the present
29 invention;

30 Example 17 illustrates the extraction and
31 characterization of "adprehumulone" in functionally useful

1 quantities from hop pellets in accordance with the present
2 invention;

3 Example 18 illustrates the preparation and
4 characterization of iso-"adprehumulone" from dried hops,
5 in functionally useful quantities in accordance with the
6 present invention;

7 Example 19 illustrates the use of iso-"adprehumulone"
8 as a foam stabilizing agent in accordance with the present
9 invention;

10 Example 20 illustrates the extraction and
11 characterization of prehumulone from hops in functionally
12 useful quantities;

13 Example 21 illustrates the preparation of iso-
14 prehumulone in functionally useful quantities; and

15 Example 22 illustrates the comparative unsuitability
16 of iso-prehumulone as a foam stabilizing agent in
17 accordance with the present invention.

18 EXAMPLE 1

19 Dihydro-alpha-acids

20 (especially Dihydro-humulone) in Hop Extract

21 Dichloromethane extracts of hops that contained the
22 water-solubles (prepared by Hops Extract Corporation of
23 America, Yakima, Washington) were analyzed by reverse-
24 phase, high-performance liquid chromatography (HPLC) at
25 20-25°C using an octadecyl, 5 µm silica (Nucleosil)
26 column. The Mobile Phase "A" was 83 parts¹¹ methanol /
27 17 parts water / 0.24 parts 85% phosphoric acid / 0.05
28 parts 0.1 M Na₄EDTA at a flow rate of 1 ml/min. The
29 absorbance of the eluants at 270 nm was continuously
30 recorded. Some extracts had an unusually high amount of a
31

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1. Throughout the specification, "parts" is to be taken
to mean "parts by volume".

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1 compound that eluted about halfway between
2 humulone+adhumulone (adhumulone co-eluted with humulone in
3 this Mobile Phase) and colupulone. One extract contained
4 1.15% of the unknown compound (assuming that it had the
5 same extinction coefficient as that of humulone), 11.8%
6 alpha-acids and 4.92% beta-acids (Fig. 1). When this
7 extract was heated anaerobically in a sealed, airtight
8 container for 4 hours at 100°C, the HPLC peak
9 corresponding to the unknown compound was observed to
10 decline whilst another unidentified peak appeared (Fig.
11 2). This latter peak had the shorter retention time,
12 being situated close behind a group of three peaks
13 representing the major iso-alpha-acids. By running
14 analyses at different wavelengths, it was determined that
15 the original unknown compound had the spectral
16 characteristics of an alpha-acid whilst the compound
17 represented by the faster running peak had the spectral
18 appearance of an iso-alpha-acid.

19 15.0 gm of the extract was extracted two times with 80
20 ml and then, subsequently, with 60 ml of hexane by shaking
21 for 20 min. and sonicating for 10 min. The hexane
22 extracts were filtered and the unknown compound
23 partitioned into aqueous phase by successive washes with,
24 firstly, 200 ml of 0.022 M NaOH (pH=10.0), followed then
25 by 150 ml of 0.01 M NaOH (pH then adjusted to 9.9 with 2 M
26 NaOH). The phases were separated by centrifugation and
27 the pH of the aqueous phase was lowered to 2.0 and this
28 phase extracted twice with dichloromethane (DCM). The
29 aqueous phase was then discarded. The DCM was removed by
30 rotary-evaporation and the weight of the resin obtained
31 was 1.81 gm. By HPLC analysis, this resin contained an
32 estimated 116 mg of the unknown compound. The resin was

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1 dissolved in 4 ml of methanol, filtered, and 0.9 ml
2 aliquots injected (a total of six injections) onto a
3 preparative HPLC column (22.5 x 250 mm, reverse-phase,
4 octadecyl, 10 μ m, Nucleosil) and eluted with 80.8 parts
5 methanol / 19.0 parts water / 0.26 parts 85% phosphoric
6 acid at 20 ml/min. The absorbance of the eluant was
7 monitored at 270 nm. The eluant fractions that contained
8 the unknown compound were collected, diluted with an equal
9 volume of water and extracted successively with 0.3 and
10 0.15 volumes of DCM. After drying with Na₂SO₄, most of
11 the DCM was removed by rotary-evaporation, the remainder
12 transferred to a weighed vial and brought to constant
13 weight under a stream of nitrogen followed by storage
14 under reduced pressure. A yellow oil was obtained with a
15 mass of 83.6 mg. HPLC analysis of the oil showed 3
16 impurity peaks, accounting for 4.3% of the total area. The
17 structure of the unknown compound was elucidated from
18 absorbance spectra, mass spectra (electron impact (EI) and
19 chemical ionization (CI)), H- and carbon 13-NMR,
20 homonuclear (H,H) and hetero-nuclear (H,C) correlation
21 spectroscopy (COSY) and determined to be dihydro-humulone.

22 EXAMPLE 2

23 Dihydro-humulone in Hop Pellets

24 100 gm of 3 year old hop pellets (stored vacuum packed
25 and refrigerated) were extracted three times with 220 ml
26 of hexane. The filtrate (Whatman No. 4 paper) was rotary-
27 evaporated to 100 ml. The hexane layer was washed with
28 250 ml of 0.1 M Na₂CO₃ to which was added 6.6 ml of 2M
29 NaOH such that the pH of the aqueous phase was
30 approximately 10. The phases were separated by
31 centrifugation. The pH of the aqueous phase was lowered
32 to 5.8 and then extracted successively with 100 ml and 2 x

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1 50 ml of dichloromethane (DCM). The pooled DCM was
2 removed by rotary-evaporation and the resin dissolved to a
3 volume of 25 ml with methanol. A total of 8.8 ml of this
4 solution, in 11 injections, was injected onto a
5 preparative HPLC column (Example 1), and the resinous
6 compounds eluted with Mobile Phase "A" (Example 1). The
7 "dihydro-humulone" peak was collected and the compounds
8 extracted twice from the eluant with DCM after addition of
9 0.7 volumes of water. The DCM phase was dried with
10 Na_2SO_4 , DCM removed by rotary evaporation and the resin
11 dissolved in methanol. The compounds that coelute with
12 dihydro-humulone (including dihydro-adhumulone) in the
13 above Mobile Phase, as well as residual humulone and
14 colupulone, were removed by preparative HPLC using the
15 Mobile Phase "B": 73 parts methanol/27 parts water/0.24
16 parts glacial acetic acid/0.27 parts of 2M Sodium
17 acetate/0.05 parts of 0.1M Na_4EDTA and the same column
18 used above. Fractions containing 'dihydro-humulone' were
19 pooled, the pH lowered with 85% phosphoric acid, and
20 extracted twice with 0.3 and 0.15 volumes of DCM. The
21 combined DCM layers were dried with Na_2SO_4 . Most of the
22 DCM was removed by rotary-evaporation, the sample
23 transferred to a weighed vial and brought to constant
24 weight. After evaporation of the DCM under a stream of
25 nitrogen, there remained 21.7 mg. of a yellow oil that was
26 determined to be 93% pure by HPLC. The 400 MHz H-NMR
27 spectrum of the oil was nearly identical to the NMR
28 spectrum of dihydro-humulone obtained from the extract in
29 Example 1, except for the frequency of two of the hydroxyl
30 protons. (The latter fact is not believed to indicate a
31 structural difference between the two compounds examined).
32 By analytical HPLC using a Mobile Phase "C" of 78 parts

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1 methanol / 22 parts water / 0.24 parts of glacial acetic
2 acid / 0.27 parts of 2M sodium acetate/0.05 parts of 0.1M
3 Na₄EDTA, the concentration of dihydro-humulone in these
4 hop pellets was estimated to be 0.10%. This value is not
5 considerably different from the value of 0.058% obtained
6 by extraction, purification and weighing.

7 EXAMPLE 3

8 Dihydro-humulone in Baled Hops

9 The hop resins were extracted successively with 400
10 ml, 2 x 200 ml and finally 120 ml of hexane from 50.0 gm
11 of freshly baled hops (1990 crop Chinook, stored under
12 refrigeration for less than 1 month). The hexane was
13 removed by rotary-evaporation and 9.11 g of resin extract
14 obtained. 6.0 g of this extract, dissolved in 25 ml
15 methanol, was eluted through a preparative HPLC column
16 (Example 1, 22 injections), a total of 16 ml injected
17 using Mobile Phase "A" (Example 1). Fractions containing
18 a peak that coeluted with dihydro-humulone were pooled,
19 extracted with DCM and rotary-evaporated. 91% of the
20 residue was rechromatographed using a Mobile Phase "D" of
21 78 parts methanol / 22 parts water / 0.24 parts of 85%
22 phosphoric acid / 0.05 parts of 0.1 M Na₄EDTA. Once
23 again, fractions containing the "dihydro-humulone" peak
24 were pooled, extracted with DCM, rotary-evaporated and the
25 residue dissolved in methanol. About 90% of this sample
26 was eluted through the same preparative HPLC column using
27 Mobile Phase "B" (Example 2). After extraction in DCM and
28 removal of the solvent from fractions containing the
29 "dihydro-humulone" peak (to which 0.1 mole/mole of
30 butylated hydroxytoluene (BHT) had been added), there
31 remained 0.36 mg of "dihydro-humulone". This residue was
32 dissolved in DCCl₃ and the H-NMR spectrum obtained at 400

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1 MHz. The NMR spectrum was very similar to that of
2 dihydro-humulone except for singlet peaks at 0.08 and 1.27
3 ppm and a very broad peak at 1.52 ppm that are not in the
4 H-NMR spectrum of dihydro-humulone. These peaks are
5 believed to be due to impurities. As further evidence, we
6 find that the relative retention times of the "dihydro-
7 humulone" peak and that of dihydro-humulone itself in two
8 Mobile Phases are identical. The same is true also for
9 the relative retention times in two Mobile Phases of the
10 corresponding peaks that appear after photo-isomerization
11 of the "dihydro-humulone" in methanol. Assuming 90%
12 recovery of "dihydro-humulone" from 50 gm of the baled
13 hops (probably an overestimate considering all of the
14 purification steps) then the calculated amount of dihydro-
15 humulone would be 0.90 mg or 0.0018% in this sample of
16 hops.

17 EXAMPLE 4

18 Dihydro-iso-humulone in Beer

19 Samples of commercial beers were degassed by careful
20 sonication of 250 ml of beer to which were added 1 drop of
21 n-octanol. After warming to room temperature, samples
22 were once again sonicated and carefully inspected to
23 ensure no octanol droplets remained. An aliquot of each
24 sample was diluted by 8 fold in 60 parts methanol / 40
25 parts water and analyzed by HPLC.

26 One of the beers, Corona, showed a peak (Fig. 3) that
27 eluted behind iso-adhumulone, and this peak had a relative
28 retention time (relative to iso-humulone) of 1.192,
29 essentially identical to the value of 1.195 previously
30 obtained for dihydro-iso-humulone in the same HPLC system.

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1 The estimated concentration of dihydro-iso-humulone in
2 this beer was 0.17 ppm, representing about 1.0% of the
3 total isomerized alpha-acids.

4 EXAMPLE 5

5 Formation of Dihydro-humulone

6 by Catalytic Hydrogenation of Alpha-acids

7 "Alpha-fraction" was prepared by adding 50% (w/w) NaOH
8 to 1984 gm of supercritical carbon dioxide extract
9 (prepared by Hops Extract Corporation of America, Inc.) in
10 4 l of de-ionized water which was rapidly stirred at 60°C
11 until reaching a pH of 7.9. The stirrer was then turned
12 off and the phases allowed to separate. The lower,
13 resinous phase was withdrawn and the aqueous phase was
14 then titrated with 10 N sulfuric acid to a pH of 2.2 and
15 the precipitated, resinous, "alpha-fraction" collected
16 after the phases had separated. This fraction contained
17 48.8% humulone (+adhumulone), 29.7 cohumulone and 7.1%
18 beta-acids. 10g of this "alpha-fraction" were solubilized
19 and diluted to 100 ml with ethanol to which was added 0.7
20 ml of 2M NaOH, and then filtered. 70 ml of the resulting
21 solution was added to 2.0 gm of 5% platinum on carbon
22 (49.5% water by weight, Escat 22, Engelhard Corp., Edison,
23 N.J.) in a high pressure Soxhlet extractor (J & W
24 Scientific) equipped with a 0-120 (0-8.4 kg/sq. cm) psi
25 pressure gauge. Following removal of air by application
26 of vacuum, hydrogen was introduced to a (closed system)
27 pressure of 80.0 psig (5.6 kg/sq. cm) and the
28 hydrogenation reaction initiated by stirring with a
29 magnetic stirring bar at room temperature. The reaction
30 was terminated after 28 hours, the pressure having
31 decreased by 6.7 psi. The catalyst was then removed by
32 centrifugation and filtration and the concentration of
33 each compound in the ethanolic solution was determined by

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1 HPLC. About 66% of the cohumulone was found to have been
2 hydrogenated, the ratio of the concentration of dihydro-
3 humulone (+ dihydro-adhumulone) to tetrahydro-humulone (+
4 tetrahydro-adhumulone) being 8.3.

5 Hydrogenation of "alpha-fraction" at 0°C instead of at
6 room temperature caused only a slight improvement in the
7 dihydro-humulone (+ dihydro-adhumulone) to tetrahydro-
8 humulone (+ tetrahydro-adhumulone) ratio at the expense of
9 needing more catalyst (see Table 1).

10 5% Palladium on carbon (Escat 111, 49.3% water,
11 Engelhard Corp.) hydrogenated alpha-acids more rapidly
12 than Platinum on carbon, though proportionally more of the
13 tetrahydro-alpha-acids were formed (see Table 1).

14 The highest ratio of [dihydro-humulone + dihydro-
15 adhumulone] ("DHH")/[tetrahydro-humulone + tetrahydro-
16 adhumulone] ("THH") was obtained with 5% platinum on
17 alumina, although the reaction proceeded more slowly than
18 with 5% platinum or carbon even though more catalyst was
19 used (see Table 1).

20 TABLE 1

21	22	23	24	25	26	27	28
		gm Cata-					
		lyst*/gm					
	pH2	"alpha-	% "DHH"\	%Cohumu-	Reac-	%Yield	
<u>Catalyst</u>	<u>(psi)</u>	<u>fraction"</u>	<u>%"THH"</u>	<u>lone hydro-</u>	<u>tion</u>	<u>of</u>	
				<u>generated</u>	<u>Time</u>	<u>"DHH"</u>	
					<u>(hr.)</u>		
26	Pt on C	80(1)	0.12	8.3	66	28	57
27	Pt on C**	80	0.30	9.3	62	25	45
28	Pt/Alumina	80	0.43	12.7	59	51	50
29	Pd on C #	60(2)	0.13	3.4	74	7	51
30	Pt on C +	40(3)	0.20	4.3	87	8	66

31 *Dry weight basis

32 **Hydrogenation reaction at 0°C

33 #3.8 ml of 6 M NaOH were added to 10 gm of "alpha-fraction"
34 and made up to 100 ml with 95% ethanol. When diluted in
35 water, the pH was 7.9.

36 +"Alpha-fraction" prepared from liquid CO₂ extract.

37 (1) 5.6 kg/sq. cm (2) 4.2 kg/sq. cm (3) 2.8 kg/sq. cm

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EXAMPLE 6Formation of Dihydro-humulone from the Solid StateReaction of Humulone Induced with Varying Amounts of NaOH

To each 2 ml glass vial were added 55 μ l of a methanolic solution containing 12.0 mg of humulone (+adhumulone), 99% pure (prepared by HPLC) and 0.3 mg BHT as an antioxidant, plus various amounts of 1.5 M NaOH in methanol. (For example, 8.8 μ l of 1.5 M NaOH to obtain a molar ratio of $[\text{NaOH}]/[\text{humulone (+adhumulone)}] = 0.4$). The methanol was removed by vacuum-evaporation at 40°C for 2 1/2 hours. Each sample was capped under nitrogen and stored in the dark at room temperature (approximately 20°C) for 7 days.

The results in Table 2 indicate that maximal formation of dihydro-humulone (+ dihydro-adhumulone) and maximal loss of humulone (+adhumulone) occur at about 0.4 moles NaOH per mole humulone (+adhumulone). There are a number of post-lupulone peaks (detected by HPLC using a Mobile Phase of 88 parts methanol/12 parts water/0.22 parts of 85% H_3PO_4 /0.05 parts 0.1 M Na_4EDTA) whose concentration is approximately equal to that of the concentration of dihydro-humulone (on the assumption of equivalent extinction coefficients at 270 nm). Another major reaction product elutes (on HPLC) immediately before cohumulone as well as two peaks close to where iso-humulone and iso-adhumulone elute. These results suggest that donation to a humulone molecule of the required two hydrogen atoms to form dihydro-humulone is provided by a neighboring alpha-acid molecule. The exact mechanism of this reaction is not known. However, without being bound by theory, it is believed that the sodium cation might form a salt linkage between, for instance, two humulone molecules, bringing the molecules in sufficiently close proximity to cause the intermolecular transfer of hydrogen atoms and thereby forming both dihydro-humulone and a dehydrogenated humulone of unknown structure. We believe

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1 molecules of this latter compound then combine to form
2 oligomeric reaction products (appearing as the post-
3 lupulone peaks on HPLC analysis).

4 Table 2

5 [NaOH]/	6 [Humulone	mg of Dihydro-	mg of Humulone
7 (+ adhumulone)]	humulone(+ dihydro-adhumulone)	+ Adhumulone	
8 0.0	0.0	12.0	
9 0.2	0.66	9.65	
10 0.3	2.52	4.97	
11 0.4	3.14	3.41	
12 0.6	2.21	5.59	
13 0.9	0.49	10.5	

14 EXAMPLE 7

15 Dihydro-humulone Formation in Presence of Various Alkalis

16 Various alkalis were tested for their ability to form
17 dihydro-humulone (+ dihydro-adhumulone) from humulone
18 (+adhumulone). Both LiOH and KOH were dissolved in
19 methanol at a concentration of 1.0 M and added to 55 μ l of
20 a methanolic solution containing 12 mg of humulone (+
21 adhumulone) and 0.3 mg of BHT contained in a 2 ml glass
22 vial. Because of the low solubility of NaHCO_3 and Na_2CO_3
23 in methanol, the methanolic solution of humulone (+
24 adhumulone) was added to solid Na_2CO_3 and NaHCO_3 and
25 sonicated until all of the alkali was dissolved. For all
26 samples, $[\text{alkali}]/[\text{humulone (+adhumulone)}] = 0.4$.
27 Methanol was removed by vacuum-evaporation at 40°C for 2
28 1/2 hours. The vials were capped under nitrogen and
29 stored for 7 days at room temperature in the dark.

30 The results (Table 3) showed that NaOH and NaHCO_3
31 caused the greatest formation of dihydro-humulone (+
32 dihydro-adhumulone) while KOH was the least effective
33 alkali tested.

<u>Table 3</u>			
		mg of Dihydro- humulone	mg of Humulone + Adhumulone
	<u>Alkali</u>	<u>(+ dihydro-adhumulone)</u>	
6	LiOH	2.46	5.50
7	NaOH	3.16	3.42
8	KOH	0.74	9.40
9	NaHCO ₃	3.26	3.34
10	Na ₂ CO ₃	1.58	7.37

EXAMPLE 8
Dihydro-humulone Formation
in "Alpha-fraction" Incubated with NaOH.
Avoidance of Undesirable Organic Solvents

Though the preceding two examples involved using methanol. and relatively pure humulone (+adhumulone), neither is necessary for this process. The methanol can be substituted with another lower alcohol, preferably ethanol, which is a substance particularly acceptable to the brewing industry.

To 27.8 gm of "alpha-fraction" (57.9% humulone + adhumulone, 28.8% cohumulone and 4.8% beta-acids (c.f. Example 5)) was added 25.0 ml of 1.06 M NaOH in 100% ethanol (Quantum Chemical Corp.) such that $[\text{NaOH}]/[\text{alpha-acids}] = 0.39$. The sample was stirred until the "alpha-fraction" was completely dissolved and then subjected to rotary-evaporation at 40°C until frothing ceased. The temperature of the water bath was then slowly increased in steps of 10°C and maintained at each step for about 30 minutes. The final temperature was 70°C. The resin became very viscous and had a deep red color. The flask containing the resin was transferred to a vacuum desiccator, flushed with nitrogen and stored at room temperature for 23 days. An aliquot was then removed for HPLC analysis. The sample contained 14.2% dihydro-humulone (+ dihydro-adhumulone), 20.4% humulone (+ adhumulone) +

1 dihydro-cohumulone, 2.8% iso-humulone (+ iso-adhumulone),
2 11.0 % cohumulone, 4.1% beta-acids and 3.1% of substances
3 eluting before the iso-alpha-acids (and assumed to have the
4 same extinction coefficients at 270 nm). The yield of
5 dihydro-humulone (+ dihydro-adhumulone) was 24%.
6 Conversion efficiency was 38% as calculated from the loss
7 of humulone (+ adhumulone), after allowing for the expected
8 contribution to peak area from co-eluting dihydro-
9 cohumulone.

10 EXAMPLE 9A

11 Dihydro-humulone Formed from "Alpha-fraction" 12 in Absence of Organic Solvents

13 Dihydro-humulone also can be formed from the "alpha-
14 fraction" and alkali without using any organic solvent as
15 indicated in this example.

16 To "alpha fraction" (72.2% humulone + adhumulone, 22.1%
17 cohumulone, 5.0% beta-acids) at 70°C was added a saturated,
18 aqueous solution of sodium dibasic phosphate (saturated at
19 85°C, solution contained 10.7 gm of sodium dibasic
20 phosphate, 0.4 moles of sodium dibasic phosphate per mole
21 of alpha acids). The sample was mixed with a blender for 2
22 minutes. The sample was then rotary-evaporated at 60°C for
23 20 minutes and then for 20 minutes at 70°C.

24 Other samples were prepared exactly as described above
25 except for the alkali. For the potassium dibasic
26 phosphate-treated sample, a saturated (at 80°C) solution,
27 containing 13.1 gm of potassium dibasic phosphate (0.4
28 moles of potassium dibasic phosphate per mole of alpha
29 acids) was used. For the potassium carbonate-treated
30 sample, 6.50 gm of anhydrous, 325 mesh potassium carbonate
31 (0.25 moles potassium carbonate per mole alpha acids) was
32 used.

33 After storage for 34 days at room temperature (20-22°C),
34 the samples were analysed by HPLC. The results are
35 presented in the table 4.

Table 4.

	%Dihydro- humulone (+dihydro -adhumulone)	% Humulone (+adhumulone)	%DHH* %Hum	% Cohumulone	% Beta- acids
Alkali					
Na ₂ HPO ₄	18.9	9.3	0.40	6.4	4.5
K ₂ HPO ₄	19.3	10.5	0.39	5.3	4.7
K ₂ CO ₃	18.6	12.2	0.40	6.1	4.3

*DHH refers to dihydro-humulone (+dihydro-adhumulone).
Hum refers to humulone (+adhumulone). The conversion efficiency was calculated from the loss of humulone (+adhumulone).

Example 9B

Dihydro-humulone Formed from "Alpha-fraction"
in Which Not All of The Alpha-Acids Have Been Protanated

1933 gm of liquid CO₂ extract (variety: Galena) was added to 5.0 liters of water at 55°C with stirring at 325 rpm. A total of 160 milliliters of 50% (w/w) of sodium hydroxide was added to bring the pH to 8.1. After standing for 1 hour, the aqueous phase was removed. To the aqueous phase was added 125 milliliters of 50% (w/w) sulfuric acid; the pH was 6.7. The sample was refrigerated overnight and 1022 gm of "alpha fraction" was collected. The yield of alpha acids from the CO₂ extract was 76%. The "alpha fraction" contained 43.3% humulone (+adhumulone), 29.8% cohumulone, 5.1% beta-acids and 0.5% dihydro-humulone.

The dihydro-humulone content of this "alpha fraction" increased to 2.0% after refrigeration for 16 days. After 7.5 months of refrigeration, the sample contained 6.1% dihydro-humulone with a conversion efficiency of 35% (c.f. Example 8).

1 Example 10
2 Dihydro-humulone Formation From Supercritical
3 CO₂ Extract

4 To 80.0 gm of a supercritical CO₂ extract (variety
5 Nugget, consisting of 44.1% humulone (+adhumulone), 15.5%
6 cohumulone, 18.8% beta-acids) at 70°C was added an
7 aqueous, saturated solution of potassium dibasic phosphate
8 (containing 9.62 gm of potassium dibasic phosphate, 0.4
9 moles of potassium dibasic phosphate per mole of alpha
10 acids). This sample was mixed with a blender for 2
11 minutes and then rotary-evaporated for about 15 minutes
12 at 60°C and 20 minutes at 70°C. After storage for 35 days
13 at room temperature (20-22°C) the sample consisted of 8.5%
14 dihydro-humulone (+dihydro-adhumulone), 20.1% humulone
15 (+adhumulone), 7.6% cohumulone and 18.0% beta-acids.

16 EXAMPLE 11
17 Preparation of Dihydro-iso-alpha-acids from
18 "Alpha-fraction" via Catalytic Hydrogenation

19 "Alpha-fraction" (14.2 gm, analysis as per Example 8)
20 was hydrogenated with 5% platinum on carbon (Johnson
21 Matthey Inc., type 18M, 51.9% water, 0.25 gm catalyst
22 per gm "alpha-fraction") in methanol at 60 psig for 4
23 hours at room temperature. Analysis showed that 92%
24 of the cohumulone had been hydrogenated and the ratio
25 [dihydro-humulone (+ dihydro-adhumulone)]/[tetrahydro-
26 humulone (+ tetrahydro-adhumulone)] was 2.9. Following
27 recovery of the catalyst by filtration, most of the
28 methanol was removed by rotary-evaporation. The resultant
29 resin was then stirred in 60 ml of deionized water
30 at 55°C and 6 M NaOH was added until a pH of 9.6 was
31 reached. 3.2 ml of 2 M MgSO₄ was then added slowly. The
32 temperature was raised to 80°C under nitrogen atmosphere
33 and maintained for 1 1/2 hours. 10 N H₂SO₄ was

1 then added until the pH was 2.1. The weight of the
2 orange-colored resinous phase thus formed was 14.3 gm.
3 The isomerized, hydrogenated resin contained:
4 Dihydro-iso-humulone 3.33 g
5 Dihydro-iso-cohumulone 2.21 g
6 Dihydro-iso-adhumulone + tetrahydro-iso-
7 cohumulone 2.13 g
8 Tetrahydro-iso-humulone
9 (+ tetrahydro-iso-adhumulone) 2.08 g
10 Dihydro-humulone (+ dihydro-adhumulone)
11 + tetra-hydro-cohumulone 0.51 g
12 Dihydro-cohumulone 0.38 g
13 Iso-humulone 0.33 g
14 Iso-cohumulone 0.26 g
15 Hydrogenated beta-acids 0.36 g
16 Tetrahydro-humulone (+ tetrahydro-
17 adhumulone) 0.12 g
18 A total of about 90% of the dihydro-humulone was
19 calculated to have been isomerized into dihydro-iso-
20 humulone.

21 EXAMPLE 12

22 Preparation of Dihydro-iso-alpha-acids from
23 CO₂ Extract via Catalytic Hydrogenation

24 20.0 gm of supercritical CO₂ extract (Eroica) was
25 dissolved to 100 ml volume with 95% ethanol plus 0.6 ml
26 of 6 M KOH. The solution was stirred with 4.0 gm of
27 activated carbon for 1 hour with nitrogen bubbled
28 through the solution. After filtration and storage
29 in a freezer, about 70 ml of the above solution was
30 added to 4.2 gm of 5% platinum on carbon (Escat 22,
31 Engelhard Corp., 49.5% water). The hop resins were
32 hydrogenated at 80 psig for 4.5 hours at room temper-
33 ature. A decrease in pressure of 14.9 psi was recorded.
34 After filtration and washing of the catalyst with

1 methanol, the volume of filtrate was brought to 100 ml.
2 HPLC analysis showed that 50.6% of the cohumulone had been
3 hydrogenated and the ratio [dihydro-humulone (+ dihydro-
4 adhumulone)] / [tetrahydro-humulone (+ tetrahydro-
5 adhumulone)] was 9.3.

6 Most of the solvent was removed from 30 ml of the above
7 solution by rotary-evaporation. 100 ml of deionized water
8 was added to the resultant resin and a total of 4.4 ml of
9 2 M KOH was added to the rapidly vortexed suspension at
10 60°C in a flask to give a final pH of 8.4. The black,
11 resinous phase was discarded after centrifugation. To the
12 aqueous solution was added 0.52 ml of 1.5 M MgSO₄ and the
13 pH raised to 9.6 with addition of 0.3 ml of 2 M KOH.
14 After incubating the solution for 1 hour at 90° C, the pH
15 was lowered to about 1.6 with 10 N H₂SO₄. After standing
16 for a while at 70°C, the translucent aqueous phase was
17 removed. The yellow resinous phase was stirred in 2 ml of
18 deionized water at 60°C and the pH brought to 8.5 - 9 with
19 addition of 6 M KOH. A clear, yellow-orange solution
20 suitable for addition to beer was obtained that consisted
21 of:

22	Dihydro-iso-cohumulone + iso-adhumulone	9.1%
23	Dihydro-iso-humulone	8.0%
24	Iso-humulone	7.6%
25	Iso-cohumulone	6.8%
26	Dihydro-iso-adhumulone + tetrahydro-	
27	iso-cohumulone	2.5%
28	Tetrahydro-iso-humulone	0.8%
29	Beta-acids	4.9%

30 About 98% of the dihydro-humulone (+ dihydro-
31 adhumulone) formed after hydrogenation was calculated to
32 have been isomerized into dihydro-iso-humulone (+ dihydro-
33 iso-adhumulone).

EXAMPLE 13Isomerization of Alkali-treated "Alpha-fraction"

"Alpha fraction" was treated with an aqueous, saturated solution of sodium dibasic phosphate as described in example 9 and was stored at room temperature for 16 days. About 185 gm of the resin was dissolved in absolute ethanol and filtered. The ethanolic solution contained 25.7 gm of dihydrohumulone (+dihydro-adhumulone). To the ethanolic solution was added 25 milliliters of 6 Normal potassium hydroxide and then all of the sample was added to 3.3 liters of de-ionized water at 69°C with stirring and under nitrogen atmosphere. A total of 134 milliliters of 1 Molar potassium hydroxide (+0.1 Molar potassium carbonate) was added slowly while the temperature of the sample was increased to 91°C; the pH was 9.7. The reaction was continued for 11.5 hours with occasional addition of alkali to maintain the pH at 9.3 to 9.5. 87% of the dihydrohumulone (+dihydro-adhumulone) was isomerized to dihydro-iso-humulone (+dihydro-iso-adhumulone). The pH was decreased to 7.6 by addition of 5% (w/w) sulfuric acid; the temperature was decreased to 76°C. The reddish-colored resin was discarded. The aqueous phase contained 15.2 gm of dihydro-iso-humulone (+dihydro-iso-adhumulone) and 0.5 gm of dihydrohumulone (+dihydro-adhumulone). The yield of dihydro-iso-humulone (+dihydro-iso-adhumulone) from the dihydrohumulone (+dihydro-adhumulone) was 59%.

EXAMPLE 14Foam Stabilizers Added to Beer

Purified compounds were obtained by preparative HPLC methodology described in Examples 1-3. Iso-cohumulone and iso-humulone were isolated from a 30% aqueous iso-alpha-acids solution (Steiner Hops Ltd., Epping, U.K.), dihydro-iso-alpha-acids from hydrogenated and isomerized "alpha-fraction" (see Example 11) and tetrahydro-iso-alpha-acids from "Tetralone" (Kalsec Inc.). The purified compounds were first dissolved in methanol (50-70 mg/ml) and then diluted in an aqueous solution of 0.015 M NaOH. Dilute NaOH was added to adjust the pH in the range of 7-8 so as to dissolve all of

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1 the compound. The final concentration of each compound,
2 about 5 mg/ml, was determined by HPLC. Aliquots were
3 added to bottles of commercial beers which were then
4 capped, swirled vigorously and stored overnight in a
5 refrigerator. An obvious ring of insoluble material was
6 observed to have formed just above the liquid surface in
7 the neck of bottles to which had been added 5 or 10 ppm of
8 tetrahydro-iso-humulone or 10 ppm of tetrahydro-iso-
9 cohumulone. Only a slight ring was formed with the
10 addition of 10 ppm of dihydro-iso-humulone and none at
11 all at 5 ppm addition. Bottles were placed in a 10°C
12 water bath for at least one hour before being tested for
13 foam stability and foam lacing.

14 In order to test foam stability, bottles of beer were
15 poured into a 1 liter, cylindrical separatory funnel with
16 the help of a wide mouth funnel. Every 165 seconds, 75 ml
17 of beer was drained from the funnel within 15 seconds,
18 until all of the beer was removed (by 10 minutes). The
19 foam was collapsed with 2 ml of added ethanol, then
20 drained from the funnel and weighed. The foam stability
21 was recorded as the ratio of the weight of the foam of the
22 sample to that of the average weight of the foam of the
23 control beers (to which corresponding amounts of water had
24 been added). Results are given in Table 5. Each value is
25 the average from at least two experiments.

26 Foam lacing was visually assessed by the following
27 procedure: the bottled beer was poured into 1 l, tall
28 form beakers (previously cleaned with chromic/sulfuric
29 acid, rinsed with deionized water and air dried) using a
30 pouring device constructed as specified by Jackson and
31 Bamforth, J. Inst. of Brewing (1982), 88, p 378. After
32 exactly 10 minutes, the beakers were photographed and the
33 lacing compared.

Table 5

Foam Stability of Beers Treated with Foam Stabilizers

Foam Stability

							Old
		Conc.	Bud-		Bud	Coors	Milwaukee
	<u>Compound</u>	<u>(ppm)</u>	<u>weiser</u>	<u>Busch</u>	<u>Light</u>	<u>Light</u>	<u>Light</u>
8	Iso-cohumulone	5	1.04	1.04	1.00	1.02	1.04
10	Iso-humulone	5	1.13	1.09	1.09	----	1.09
13	Dihydro-iso-cohumulone	5	1.08	1.10	1.13	1.11	1.08
16	Dihydro-iso-humulone	5	1.16	1.16	1.22	1.35	1.31
19	Tetrahydro-iso-cohumulone	5	1.21	1.20	1.29	1.28	1.34
22	Tetrahydro-iso-humulone	5	1.41	1.32	1.36	1.53	1.49
24	"Tetralone"*	5	1.20	----	----	----	----
26	Iso-cohumulone	10	1.02	1.04	----	----	----
28	Iso-humulone	10	1.12	1.07	1.04	----	----
31	Dihydro-iso-cohumulone	10	1.21	1.08	1.14	----	----
34	Dihydro-iso-humulone	10	1.41	1.35	1.41	----	----
37	Tetrahydro-iso-cohumulone	10	1.44	1.37	1.53	----	----
40	Tetrahydro-iso-humulone	10	1.79	1.75	1.87	----	----
44	Average weight of control foam (in gm)		6.16	6.74	4.55	3.73	6.28
47	Concentration of iso-alpha-acids in						

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1 control beers
2 (ppm) 9.3 13.0 13.6 12.1 15.6
3 *Kalsec, Inc.
4 Observations:

5 In most cases dihydro-iso-humulone, at a concentration
6 of 5 ppm, significantly improved the foam stability of the
7 light beers as compared with the improvement caused by 5
8 ppm of added iso-humulone. Dihydro-iso-humulone is only
9 slightly less effective than tetrahydro-iso-cohumulone or
10 "Tetralone" (which contains predominantly tetrahydro-iso-
11 cohumulone) at a concentration of 5 ppm in most of the
12 beers tested. At 10 ppm in beer, dihydro-iso-humulone was
13 considerably more effective than iso-humulone at improving
14 the foam stability and nearly as effective as tetrahydro-
15 iso-cohumulone.

16 It was observed that in Budweiser and Bud Light beers,
17 addition of 5 ppm dihydro-iso-humulone improved foam
18 lacing to an even greater extent than did addition of 10
19 ppm iso-humulone. Both dihydro-iso-humulone and tetra-
20 hydro-iso-cohumulone effected especially obvious and
21 comparable improvements to foam lacing in all beers,
22 dependent on the quantity added.

23 EXAMPLE 15

24 Preparation of Hydrogenated, Isomerized Resin Extract (IRE)

25 Supercritical carbon dioxide extract (20.0 gm, 33.2%
26 humulone + adhumulone, 20.2% cohumulone, 28.7% beta-acids)
27 was dissolved to 100 ml volume with 95% ethanol and 2.0 ml
28 of 2M NaOH. The solution was stirred with 4.0 gm of
29 activated carbon for 1 hour with nitrogen bubbled through
30 the solution. After filtration, 70 ml of the above solution
31 was added to 7.33 gm of 5% platinum on carbon (Johnson
32 Matthey, type 18M, 51.9% water) and the mixture hydrogenated
33 at 60 psi for 17 hours. The catalyst was then removed by
34 filtration. 30 ml of water was added to the ethanolic

1 solution along with 5.86 ml of 1.45 M MgSO_4 . The pH was
2 adjusted to 8.7 by addition of 6 M NaOH. After reaction at
3 86°C for 1 hour, 10 N H_2SO_4 was added until a pH of 1.7 was
4 obtained. The resinous phase, 11.4 gm (hydrogenated IRE)
5 was collected, analyzed by HPLC and found to contain:

	<u>%</u>
6	
7	
8 dihydro-iso-humulone (+ dihydro-	
9 iso-adhumulone)	20.3
10 dihydro-iso-cohumulone*	12.3
11 tetrahydro-iso-humulone (+ tetrahydro-	
12 iso-adhumulone)	12.0
13 tetrahydro-iso-cohumulone	7.2
14 dihydro-humulone (+ dihydro-adhumulone)**	0.65
15 dihydro-cohumulone	0.71
16 tetrahydro-humulone (+ tetrahydro-adhumulone)	0.24
17 iso-humulone	0.91
18 iso-cohumulone	0.81

19
20 *includes iso-adhumulone

21 **includes tetrahydro-cohumulone

22
23 The inferred yield of dihydro-iso-humulone (+ dihydro-
24 iso-adhumulone) was about 61%.

25 Example 16

26 Brewing Trial with Hydrogenated IRE

27 Hydrogenated IRE from Example 15, 0.361 gm, was
28 dissolved in 3.0 ml of 95% ethanol plus 0.50 ml of 2M NaOH
29 (1 equivalent of NaOH per iso-alpha-acids). 169 ml of an
30 unhopped ale wort (S.G. 1.051) was boiled for 20 min.
31 before adding 0.165 ml of the hydrogenated IRE solution.
32 Boiling was continued for 15 min. and the wort then cooled
33 to room temperature and the volume adjusted back to 169 ml
34 with deionized water. The wort was centrifuged and the
35 supernatant collected. A simulated "beer" was prepared
36 from an aliquot of this wort by lowering the pH to 3.9 and
37 allowing to chill overnight in a refrigerator. Samples of
38 both the boiled wort and the "beer" were filtered, with
39 the aid of added celite (Hyflo supercel) plus silica gel,
40 through a glass

1 fiber filter (Gelman Type A-E). Before analysis by HPLC,
2 samples were diluted in methanol, sonicated and clarified
3 by syringe filtration, again through a glass fiber filter
4 (Gelman Type A-E). Yields of hydrogenated iso-alpha-acids
5 are given in the the following table.

6 Table 6

7 <u>Compound</u>	8 <u>initial conc.</u> <u>in wort (ppm)</u>	9 <u>%yield in</u> <u>boiled wort</u>	10 <u>%yield</u> <u>"in beer"</u>
11 dihydro-iso-cohumulone	9.1	84	81
12 dihydro-iso-humulone +	11.0	69	61
13 dihydro-iso-adhumulone			
14 tetrahydro-iso-cohumulone	9.4	70	63
15 tetrahydro-iso-humulone	6.2	57	45

16 EXAMPLE 17

17 Extraction of "Adprehumulone" from Stabilized Hop Pellets

18 To 256 gm of pre-ground MgO-stabilized hop pellets (3
19 years old, 1987 Nugget variety, stored vacuum packed and
20 refrigerated) were added 154 ml of 85% phosphoric acid.
21 The hops were extracted successively with 700 ml and 350
22 ml aliquots of hexane. The solvent was separated by
23 filtration and the aliquots combined. The volume of the
24 hexane solution was reduced to 250 ml by rotary-
25 evaporation and the solvent washed twice at pH 2.2-2.5
26 with 600 ml acidified water (HCl), followed by a wash at
27 pH 8.4 (600 ml of water + NaOH). The hexane layer was
28 next extracted at pH 9.8 with 500 ml of 0.026 M NaOH;
29 phases were separated by centrifugation. The aqueous
30 phase was acidified (pH = 5.7) and extracted twice with
31 DCM (saturated NaCl solution was added to help separate
32 the phases). Most of the DCM was removed by rotary-
33 evaporation. The resultant resin was dissolved in
34 methanol and aliquots eluted through a preparative HPLC
35 column (see previous Examples 1-3) using a Mobile Phase
36 "E" of 85 parts methanol / 15 parts water and 0.26 parts
37 of 85% phosphoric acid.

1 The eluant fractions containing colupulone and
2 "adprehumulone" were collected. The pooled fractions were
3 extracted and rotary-evaporated as indicated in Example 1.
4 The residue was dissolved in 25 ml hexane, then placed in
5 a freezer and the colupulone crystals that formed were
6 subsequently removed by filtration. Most of the hexane
7 was removed, first by rotary-evaporation and then with a
8 stream of nitrogen. The residue, 0.167 gm, was dissolved
9 into 1.4 ml of methanol and two aliquots injected into a
10 preparative HPLC column using Mobile Phase "C" (Example
11 2). The "adprehumulone" eluted well before colupulone in
12 this Mobile Phase. Pooled fractions containing
13 "adprehumulone" were further acidified with phosphoric
14 acid, extracted with DCM and brought to constant weight
15 under a stream of nitrogen as described in Example 1.
16 37.2 mg of a viscous, yellow resin were obtained. When
17 run on HPLC with Mobile Phase "A" (see Example 1) the
18 major constituent of this resin had almost the same
19 retention time as that of colupulone. By running samples
20 in different Mobile Phases, the major constituent compound
21 was deduced to have a purity of about 76%.

22 The carbon-13 NMR spectrum of this unknown compound (in
23 DCCl_3) revealed that it had 22 carbon atoms (chemical
24 shifts referenced to tetramethylsilane and reported in
25 table 7). The 400 MHz H-NMR spectrum shows 2 alkenic
26 protons, 4 singlet methyl peaks, 1(or possibly 2)doublet
27 methyl peaks, and two broad, presumably hydroxyl protons.
28 By homonuclear (H,H) COSY and heteronuclear (H,C) COSY,
29 the assignments of the protons and the carbon atoms were
30 determined (see Table 7 below).

31 Table 7

32 Proton and Carbon-13 Chemical Shifts of

33 "Adprehumulone" in DCCl_3

34 Chemical Shift (ppm)

35 <u>Carbon No.</u>	<u>C</u>	<u>H</u>
----------------------	----------	----------

1				
2	1	13.93	0.90, 0.92	(CH ₃)
3	2	17.75	1.72	(CH ₃)
4	3	17.86	1.52	(CH ₃)
5	4	21.05	3.06	(CH ₂)
6	5	22.44	1.34	(CH ₂)*
7	6	25.30	1.58	(CH ₂)**
8	7	25.73	1.68	(CH ₃)
9	8	25.98	1.69	(CH ₃)
10	9	31.65	1.36	(CH ₂)*
11	10	37.92	2.87	(CH ₂)
12	11	42.63	2.42, 2.57	(CH ₂)
13	12	78.73		
14	13	105.49	----	
15	14	109.49	----	
16	15	115.72	5.00	(CH)
17	16	120.97	5.12	(CH)
18	17	132.81	----	
19	18	138.24	----	
20	19	167.66	----	
21	20	190.72	----	
22	21	194.97	----	
23	22	200.76	----	

24 * Shifts on carbon nos. 5 & 9 are believed to be due to two
 25 CH₂ groups, but it is possible that spectral lines are due to
 26 a CH₃ and a CH group.

27 ** The number of protons was not precisely established.
 28

29 EXAMPLE 18

30 Preparation of Iso-"adprehumulone" From Dried Hops

31 260 gm of freshly ground, dried hops (about 7 months
 32 old, Nugget variety) were extracted successively with 1600
 33 ml and 700 ml of hexane. The hexane was collected by
 34 filtration, rotary-evaporated down to 250 ml and re-
 35 filtered. It was then shaken with 600 ml of 0.1 M Na₂CO₃,
 36 30 ml of 1M HCl being added to adjust the pH to 8.3 at
 37 equilibrium. The aqueous phase was discarded and the
 38 hexane layer washed with 600 ml of 0.05 M Na₂CO₃,
 39 adjusting the pH to 9.7 with 1.5 ml of 2M NaOH.
 40 After standing for 1 hour, the phases were separated and
 41 the pH of the aqueous phase adjusted to 2.7. This phase
 42 was then extracted successively with 75 ml and 35 ml of
 43 DCM. (Some

1 saturated NaCl solution was added to effect a good phase
2 separation). Most of the DCM was removed by rotary-
3 evaporation followed by a stream of nitrogen. The
4 resinous product was solubilized by the addition of 7 ml
5 of methanol. A total of 14.2 ml of this solution (about
6 85% of the sample) was injected onto the preparative HPLC
7 column and eluted using Mobile Phase "E" (Example 17).

8 To pooled fractions containing colupulone and
9 "adprehumulone" (=1.00 volume) were added 0.61 volumes of
10 water and 0.2 volumes of DCM. The phases were shaken,
11 separated and the DCM layer dried over Na₂SO₄ before
12 rotary-evaporation. The residue (0.59 gm) was dissolved
13 in 30 ml of methanol. Aliquots of this solution were
14 transferred to glass tubes and placed outside in direct
15 sunlight to effect photo-isomerization.

16 15 ml of DCM and then 40 ml of water were added to the
17 recombined solutions and the phases shaken together and
18 separated. The aqueous phase was extracted a second time
19 with 10 ml of DCM. The DCM layers were combined and the
20 solvent removed by rotary evaporation. The residue was
21 dissolved in methanol and most of the sample injected onto
22 a preparative HPLC column and eluted with Mobile Phase "D"
23 (Example 3). Eluant fractions containing photo-iso-
24 "adprehumulone" were shaken with 0.33 volumes of DCM and
25 0.78 volume of water. Most of the collected DCM was
26 removed by rotary-evaporation. After transfer to a
27 weighed vial, the remaining solvent was removed, firstly
28 with a stream of nitrogen and then under vacuum. Mass of
29 the residual resin was 48.4 mg. The apparent purity of
30 the sample, obtained by HPLC with Mobile Phase "A"
31 (Example 1), was 91%. HPLC using a Mobile Phase of 72.5
32 parts methanol / 26.1 parts water / 0.725 parts of 85%
33 phosphoric acid / 0.483 parts of 0.2 M Mg acetate / 0.24
34 parts of 0.1 M Na₄EDTA that separates cis- and trans-
35 forms of the major iso-alpha-acids; showed one major peak,

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1 presumed to be the trans-iso-"adprehumulone", at an
2 estimated purity of 92%.

3 The CI (isobutane) MS of photo-iso-"adprehumulone" had
4 a base peak at an m/z of 377, indicating a molecular
5 weight of 376.

6 Before analysis by NMR spectroscopy, 0.17 mg of BHT was
7 added to 4.0 mg. of photo-iso-"adprehumulone". The 400
8 MHz H-NMR spectrum of photo-iso-"adprehumulone" (in DCCl₃)
9 had spectral peaks with the following chemical shifts
10 (ppm): 0.88, 0.90 (CH₃), 1.32 (CH₂, CH₂), 1.53 (CH₃), 1.57
11 (CH₃), 1.67 (CH₂?), 1.69 (CH₃), 1.73 (CH₃), 2.32 and 2.56
12 (CH₂), 2.83 (CH₂), 3.05 (CH), 3.30 (CH₂), 5.12 (CH), 5.19
13 (CH).

14 EXAMPLE 19

15 Foam Stabilizing Effect of Photo-iso-"adprehumulone" in Beer

16 An aqueous solution of 3.55 mg/ml photo-iso-
17 "adprehumulone" was prepared by adding 0.40 ml of 50 mg/ml
18 of photo-iso-"adprehumulone" (Example 18), in methanol, to
19 5.0 ml of 0.012 M NaOH plus 0.23 ml of water. The pH was
20 8.5-9.0. "Tetralone" (Kalsec Inc., 20% in propylene
21 glycol) was diluted in deionized water and the pH brought
22 to 8.5 with 2 M NaOH.

23 The aqueous solutions of photo-iso-"adprehumulone" and
24 "Tetralone" (also at 3.55 mg/ml) were added to Budweiser
25 beer, foam stability tested as described in Example 14,
26 and the results recorded in Table 8.

27 Photo-iso-"adprehumulone" significantly improved the
28 foam stability of beer in a concentration dependent
29 manner.

30 Table 8

31 Foam Stability of Beer Containing Photo-iso-"adprehumulone"

32	33	34	35
	<u>Sample</u>	<u>Concentra-</u> <u>tion (ppm)</u>	<u>Foam Stability of</u> <u>Beer (relative to</u> <u>control)</u>
36	photo-iso-"adprehumulone"	5	1.12
37	photo-iso-"adprehumulone"	8	1.33
38	"Tetralone"*	5	1.20

1 "Tetralone"* 8 1.45

2
3 *Kalsec, Inc.

4 Example 20

5 Prehumulone Isolated From Hops

6 225 gm of hops (Chinook, 1990 crop) were extracted
7 successively with 2 l and 1 l of hexane. After
8 filtration, the combined volume of the hexane solution was
9 reduced to 220 ml by rotary-evaporation. As determined by
10 HPLC, the hexane solution contained an estimated 103 mg of
11 a presumed alpha-acid that had a relative retention time
12 of 1.325 (relative to humulone (+ adhumulone) using Mobile
13 Phase "A"). The hexane solution was then washed with 600
14 ml of 0.025 Na₂CO₃ (pH of the aqueous phase= 7.6). The
15 hexane phase was collected and successively washed with
16 600 ml aliquots of 0.046M NaOH (aqueous phase pH = 8.2)
17 and then 0.037M NaOH (aqueous phase pH = 9.4), the phases
18 being separated by centrifugation. Both aqueous phases
19 were combined and the pH lowered to 3.0 with 10 N H₂SO₄.
20 The aqueous phase was extracted with DCM until all of the
21 color was transferred into the DCM (saturated NaCl was
22 added to help break the phases). After rotary-evaporation
23 of the DCM, the 10.4 gm of collected resin was dissolved
24 in methanol to a final volume of 25 ml. This solution
25 contained an estimated 45 mg of "prehumulone". A total of
26 20.7 ml of the methanolic solution (23 injections) were
27 eluted through a preparative HPLC column using Mobile
28 Phase "E" of Example 17. Fractions containing the
29 "prehumulone" were pooled and then extracted with DCM,
30 substantially as described in Examples 1-3. The remaining
31 resin, after rotary-evaporation, was dissolved in 10 ml of
32 hexane and chilled in a freezer overnight. The resultant
33 colupulone crystals were removed by filtration. The
34 hexane was evaporated with a stream of nitrogen and the
35 resin, containing an estimated 26 mg of "prehumulone", was

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1 dissolved in a little methanol. The "prehumulone" in this
2 methanolic solution was further purified by preparative
3 HPLC using mobile phases "D" and "A". Pooled fractions
4 containing purified "prehumulone" were extracted into DCM
5 in the presence of BHT and dried over Na₂SO₄. The DCM was
6 removed by rotary-evaporation and the sample brought to
7 constant weight under a stream of nitrogen. 15.6 mg of a
8 viscous, yellow resin was obtained (excluding the mass of
9 BHT). HPLC analysis indicated a purity of about 92%.

10 NMR analysis was performed on a sample of the resin
11 dissolved in DCCl₃. From the ¹H and carbon-13 NMR spectra
12 and the heteronuclear (H,C) COSY spectrum the carbon and
13 proton assignments, as listed in Table 9, were obtained.
14 The carbon assignments for the hydroxyl protons at 4.21
15 and 7.05 ppm and the enolic proton at 18.82 ppm could not
16 be made.

17 Table 9

18 Carbon-13 and Proton NMR Data of Prehumulone.

19 Chemical Shifts (ppm)

20	21	22	23
	¹³ C	¹ H	Number of Protons
24	17.74	1.73	3
25	17.86	1.53	3
26	21.05	3.04, 3.08	2
27	22.31	0.94	3
28	22.36	0.93	3
29	25.71	1.68	3
30	25.96	1.69	3
31	28.11	1.64	1
32	34.48	1.47, 1.60	2
33	36.07	2.87	2
34	42.64	2.44, 2.54	2
35	115.76	5.00	1
36	120.99	5.12	1
37	Other carbon-13 chemical shifts include: 78.75, 105.44,		
38	109.17, 132.77, 138.17, 167.67, 190.72, 194.97, and 201.06		
39	ppm.		

1 From the homonuclear (H,H) COSY, the molecular
2 connectivities of the hydrogen-containing carbons were
3 established. The R group was tentatively identified as
4 $-\text{CH}_2 -\text{CH}_2 -\text{CH} -(\text{CH}_3)_2$. An alpha-acid having this same
5 structure has previously been identified as prehumulone by
6 Rillaers, G. and Verzele, M. in Bull. Soc. Chim. Belg.
7 (1962) 71, 438-445.

8 Example 21

9 Preparation of Photo-iso-prehumulone

10 After NMR analysis, DCCl_3 was removed from the
11 prehumulone solution (Example 20) and the sample was then
12 redissolved in 1.0 ml of methanol. The prehumulone was
13 then photo-isomerized by exposure to sunlight. The photo-
14 iso-prehumulone so formed was purified by preparative HPLC
15 using Mobile Phase "D" of Example 3 and extracted into DCM
16 as described in Examples 1-3. After rotary-evaporation of
17 the DCM, the sample was brought to constant weight with a
18 stream of nitrogen. 5.4 mg of the photo-iso-prehumulone
19 obtained was dissolved in 0.17 ml of 95% ethanol and then
20 1.4 ml of 0.01 M NaOH was added. The solution had a pH of
21 about 9.2 and was estimated to be 99% pure by HPLC. The
22 concentration of photo-iso-prehumulone was 3.4 mg/ml.

23 Example 22

24 Photo-iso-prehumulone as a Foam Stabilizer In Beer.

25 Aqueous solutions of photo-iso-prehumulone (Example
26 21), dihydro-iso-humulone and tetrahydro-iso-cohumulone
27 were added to bottles of Bud Light beer which were then
28 capped, swirled vigorously and stored overnight in a
29 refrigerator. The concentration of each compound in the
30 beer was 5 ppm. Foam stability was tested as described in
31 Example 14. Results (Table 10) indicated that photo-iso-
32 prehumulone is not an especially effective foam
33 stabilizer. Comparison with Table 5, shows that photo-
34 iso-prehumulone is no more effective as a foam stabilizer
35 than iso-humulone.

1

Table 10

2

Foam Stability of Beer Treated with Compounds

3

at a Concentration of 5 ppm*

4

Compound

5

Foam Stability

6

Photo-iso-prehumulone

1.05

7

Dihydro-iso-humulone

1.28

8

Tetrahydro-iso-cohumulone

1.30

9

10

*Results are an average from two bottles of Bud Light beer.

11

12

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1 Utility of the Invention

2 As can be seen from the foregoing, dihydro-iso-humulone
3 or iso-"adprehumulone" advantageously may be employed as
4 the sole or major ingredient of foam stabilizing
5 preparations. Moreover, we have observed that dihydro-
6 iso-humulone containing preparations in accordance with
7 the present invention appear to be less prone to "bath-tub
8 ring" than are tetrahydro-iso-alpha-acids employed at
9 similar concentrations. In addition, the lower bittering
10 potential of dihydro-iso-humulone allows for greater
11 flexibility in use without necessitating such substantial
12 changes in brewery hopping procedures in order to enhance
13 beer foam to the desired extent.

14 It is a further advantage of our invention that the
15 brewer is enabled to enhance beer foam through use of
16 isomerized derivatives of substances that are naturally
17 occurring compounds found in quite minor amounts in fresh
18 hops, and in the case of dihydro-humulone in somewhat
19 greater amounts in hop pellets, and observed to exist in
20 hop extracts. Quite minor (functionally insignificant)
21 amounts of the dihydro-iso-humulone are believed to have
22 been observed in certain commercial beers.

23 The dihydro-iso-humulone or iso-"adprehumulone" in
24 accordance with the present invention may be added to beer
25 over a wide range of concentrations depending on taste.
26 Typically from about 2 to 30 ppm, preferably 5 to 10 ppm,
27 depending on foam stabilization and taste desired, has
28 been found to be adequate. The dihydro-iso-alpha-acids or
29 iso-"adprehumulone" in accordance with the present
30 invention may be added during brewing or following
31 brewing. Various changes may be made in the invention
32 without departing from the spirit and scope thereof. It
33 is therefore intended that the invention not be limited by
34 the foregoing description.

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1 THE EMBODIMENTS OF THE INVENTION IN WHICH AN
2 EXCLUSIVE PROPERTY OF PRIVILEGE IS CLAIMED ARE DEFINED
3 AS FOLLOWS:

4 1. A hops-derived foam stabilizing preparation for a beverage,
5 characterized by at least one dihydro-iso-alpha acid, selected from dihydro-
6 iso-humulone, dihydro-iso-adhumulone and mixtures thereof, as a principal
7 stabilizing agent.

8 2. A hops-derived foam stabilizing preparation for a beverage,
9 characterized by comprising iso-adprehumulone as a principal stabilizing
10 agent.

11 3. In a hops-flavored beverage, characterized by the addition of at least
12 one dihydro-iso-alpha acid selected from dihydro-iso-humulone, dihydro-iso-
13 adhumulone and mixtures thereof, as a foam stabilizing and bittering agent.

14 4. In a beverage according to claim 3, characterized in that the
15 dihydro-iso-alpha-acid is added in an amount in the range of from about 2 to
16 about 30 ppm.

17 5. In a hops-flavored beverage, the improvement characterized by the
18 addition of iso-adprehumulone in an amount in the range of from about 2 to
19 about 30 ppm, as a foam stabilizing and bittering agent.

20 6. In a method of preparing a malt beverage in which wort is prepared
21 and fermented, and a foam stabilizing and bittering agent added, the
22 improvement characterized in that the foam stabilizing and bittering agent
23 comprises at least one dihydro-iso-alpha-acid selected from dihydro-iso-
24 humulone, dihydro-iso-adhumulone and mixtures thereof.

25 7. In a method according to claim 6, characterized in that the dihydro-
26 iso-alpha-acid is added in an amount in the range of from about 2 to about 30
27 ppm.

28 8. In a method according to claim 6, characterized in that the dihydro-
29 iso-alpha-acid is added prior to, during or following fermentation.

1 9. In a method of preparing a malt beverage in which wort is prepared
2 and fermented, and a foam stabilizing and bittering agent added, the
3 improvement characterized in that the foam stabilizing and bittering agent
4 comprises iso- α -humulone which is added in an amount in the range of
5 from about 2 to about 30 ppm.

6 10. In a method according to claim 9, characterized in that iso-
7 α -humulone is added prior to, during or following fermentation.

8 11. A process for the preparation of one or more dihydro- α -acids
9 characterized by the reaction of corresponding α -acids in the presence of
10 about 0.3 to about 0.6 equivalents of alkali per mole of α -acids in a
11 substantially non-aqueous environment.

12 12. A process according to claim 11, characterized in that the reaction
13 is conducted in the presence of about 0.4 equivalents of alkali per mole of
14 α -acids.

15 13. A process according to claim 11, characterized in that the alkali is
16 selected from NaOH, LiOH, KOH, Na_2CO_3 , NaHCO_3 , Na_2HPO_4 and K_2CO_3 .

17 14. A process according to claim 11, characterized in that the reaction
18 is conducted below about 80°C.

19 15. A process according to claim 11, characterized by the step of
20 dissolving said alkali in a lower alcohol or water, and adding the resulting
21 solution to said α -acids.

22 16. A process according to claim 15, characterized in that the lower
23 alcohol comprises a C_1 to C_4 alcohol.

24 17. A process according to claim 11, characterized by the step of
25 extracting hops to prepare said α -acids prior to said reaction.

26 18. A process according to claim 17, characterized in that the hops are
27 extracted using carbon dioxide.

28 19. A process according to claim 18, characterized in that the hops are
29 extracted using supercritical carbon dioxide, or liquid carbon dioxide.

1 20. A process according to claim 17, characterized by the step of
2 pelletizing said hops prior to said extracting.

3 21. A process for the preparation of dihydro-alpha acids and
4 tetrahydro-alpha-acids in a ratio of in excess of 4.3:1 by catalytic hydrogenation
5 of alpha-acids in the presence of a platinum/alumina catalyst or a platinum/carbon
6 catalyst.

7 22. A composition containing dihydro-iso-alpha acids and
8 tetrahydro-alpha-acids in a ratio of 4.3-12.7 to 1.0 when made by the process of
9 claim 21.

10 23. A process for producing a foam stabilizing and bittering agent for
11 a malt beverage characterized by extracting pelletized hops under conditions
12 which favor the separation and recovery of a fraction rich in adprehumulone,
13 and subjecting the adprehumulone fraction to photo-isomerization to produce
14 iso-adprehumulone.

15 24. A process for producing a foam stabilizing and bittering agent for
16 a malt beverage characterized by the steps in sequence of: (1) extracting hops
17 under conditions which favor the separation and recovery of a fraction rich in
18 alpha-acids; (2) subjecting the alpha-acids fraction to selective hydrogenation
19 in the presence of a platinum/alumina catalyst or a platinum/carbon catalyst, to
20 produce dihydro-alpha-acids; and (3) isomerizing said dihydro-alpha-acids so
21 as to form dihydro-iso-alpha-acids.

22 25. A process according to claim 24, characterized by including the
23 step of pelletizing said hops prior to said extracting.

24 26. A process according to claim 24, characterized in that the hops are
25 extracted using CO₂, and including the step of isomerizing said CO₂ extract
26 following hydrogenation.

27 27. A process according to claim 26, characterized in that the hops are
28 extracted using supercritical carbon dioxide or liquid carbon dioxide.

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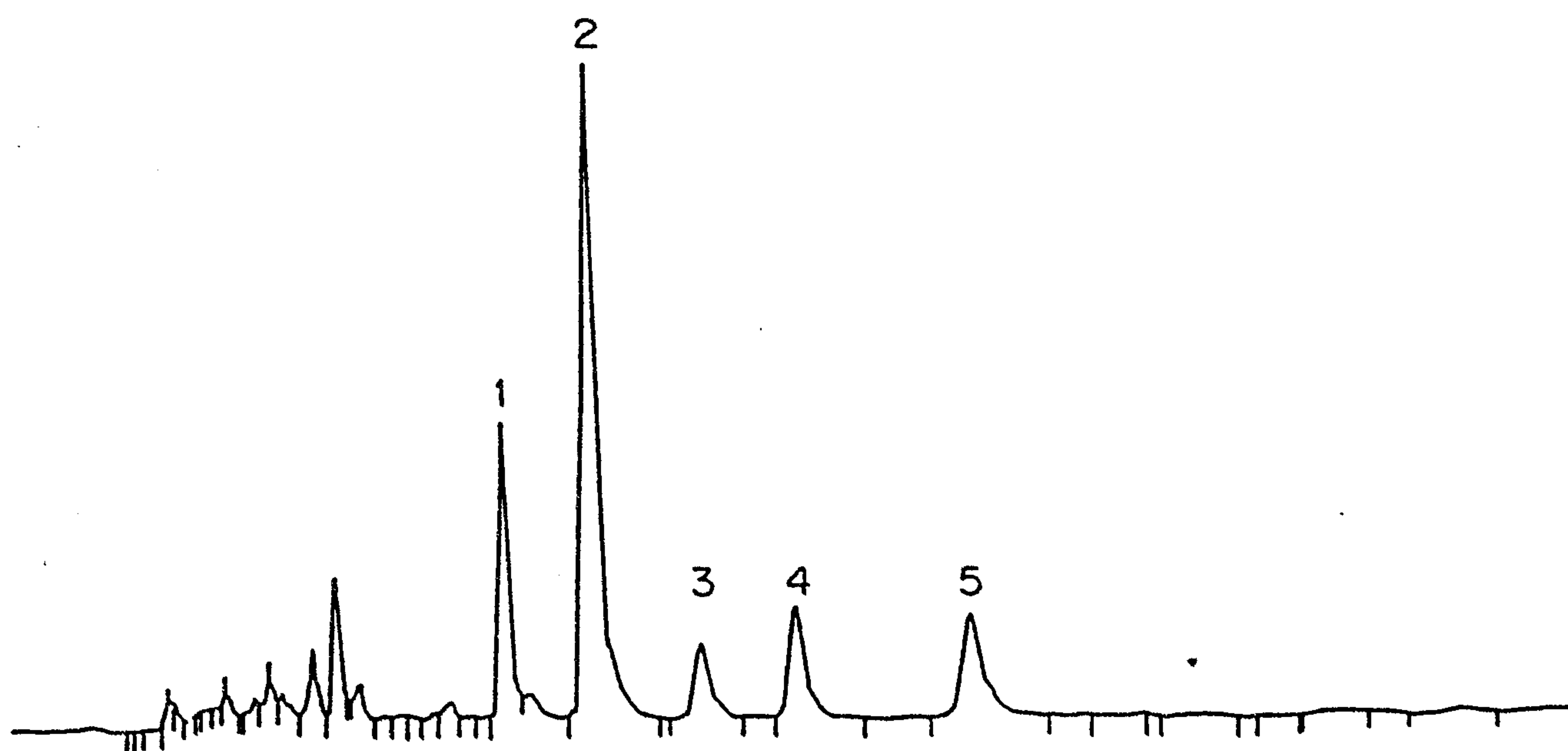


FIG.1

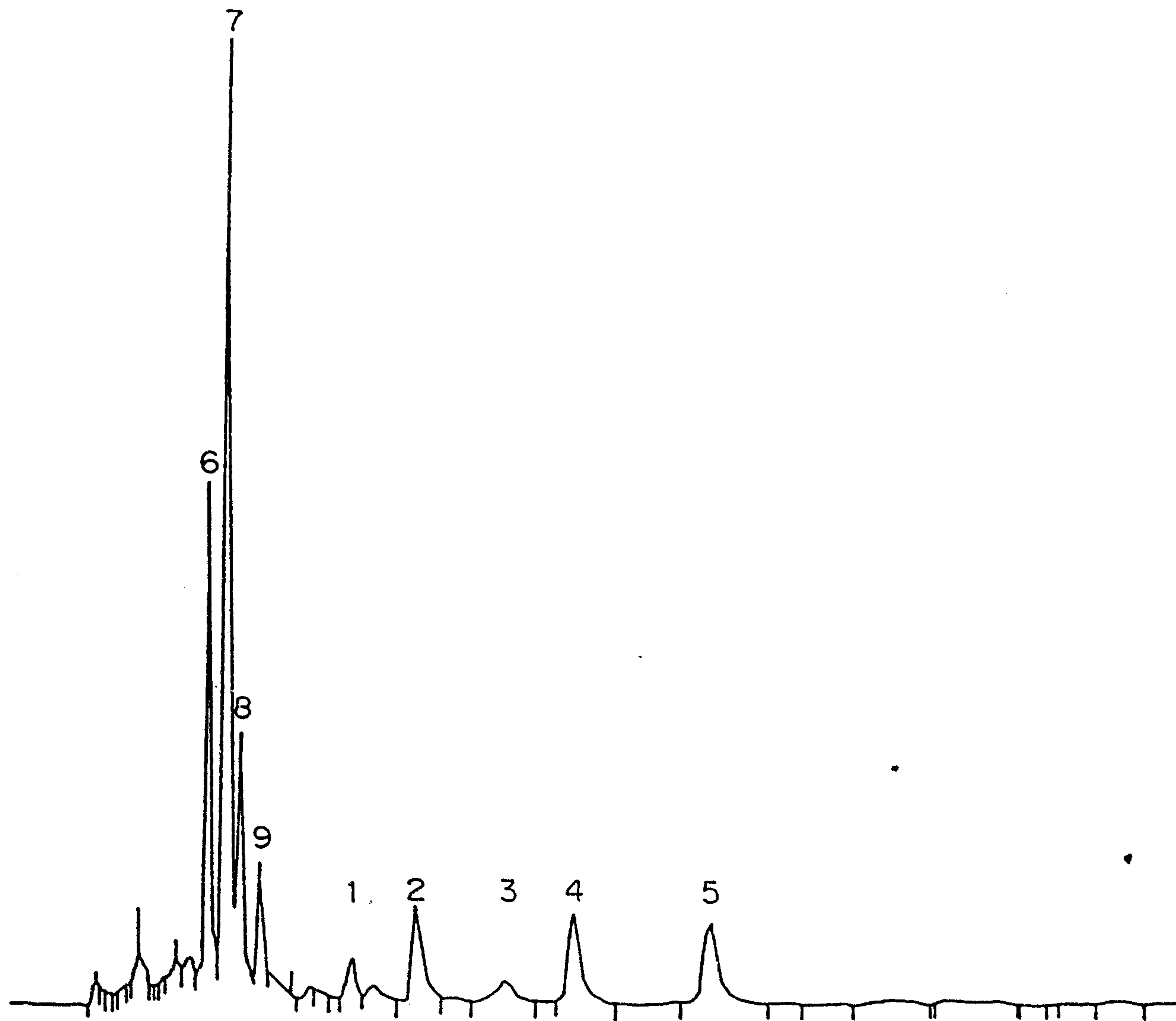


FIG.2

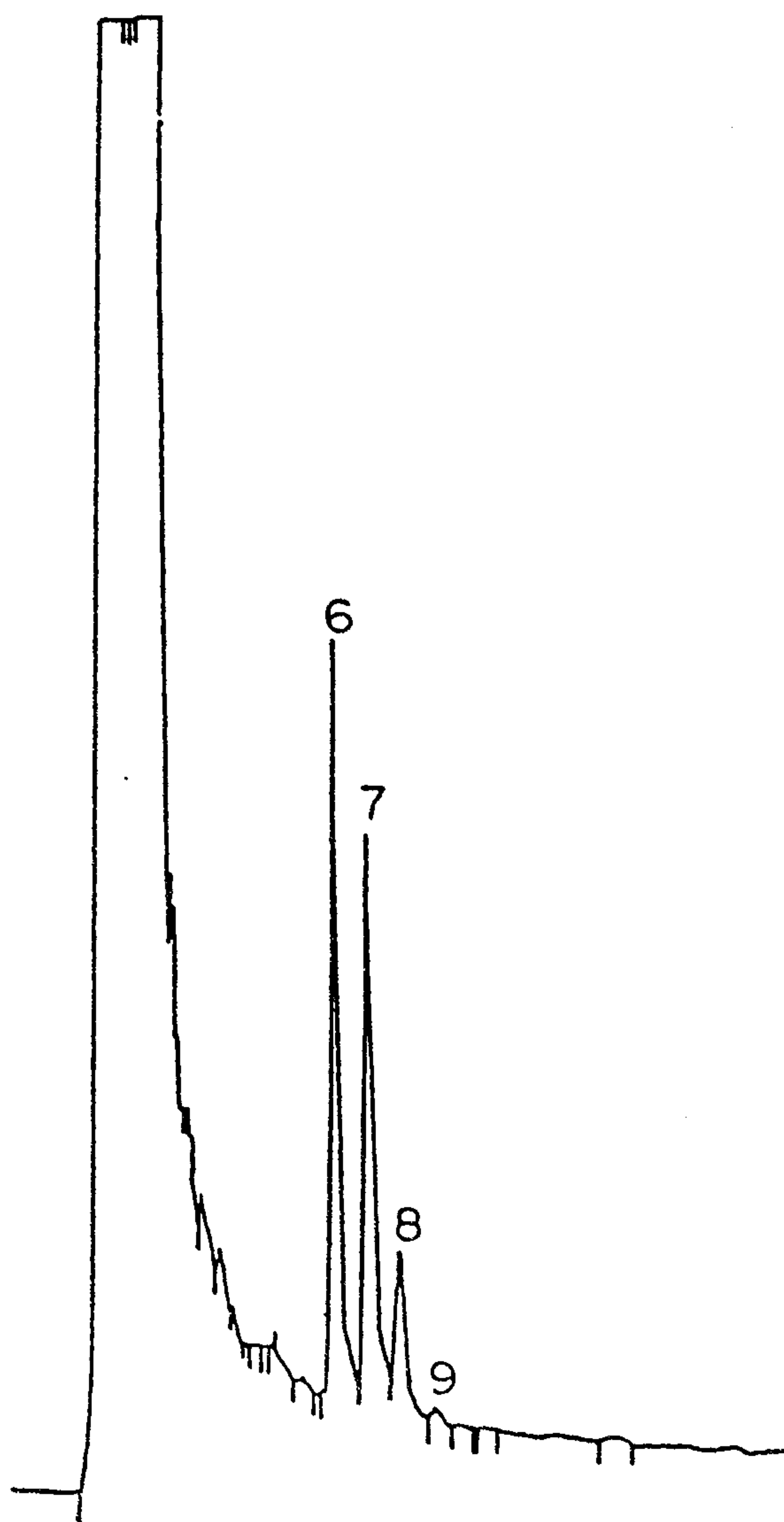


FIG.3

