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Preparation of pyrroloindole derivatives

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DESCRIPTION

PREPARATION OF PYRROLOINDOLE DERIVATIVES

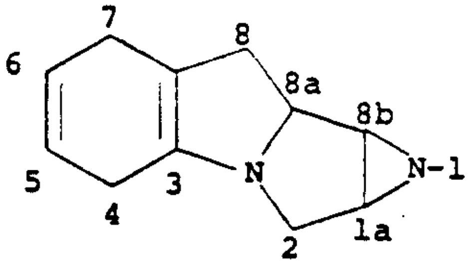
1. Field of Invention.

This Application has been divided out from our Application No.85.21816 (2164038), referred to below as our "primary" Application.

The invention of our primary Application provides novel mitomycin analogs containing a disulfide group, and processes for the preparation thereof. These compounds are mitomycin A analogs in which the 7-alkoxy group bears an organic substituent incorporating a disulfide group. The present invention provides a method of producing mitomycin A and derivatives thereof. Mitomycin A is an antibiotic of established utility, and the 7-O-substituted mitosane analogs thereof have similar utility.

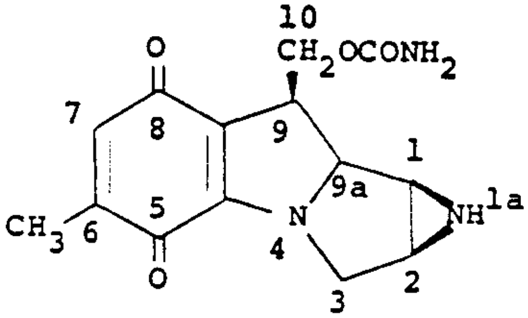
Nomenclature - The systematic Chemical Abstracts name for mitomycin A based on the recent revision [Shirhata et al., J. Am. Chem. Soc., 105, 7199 (1983)] is:

[1aS-(1aβ,8β,8aα,8bβ)]-8-[((aminocarbonyl)oxy)methyl]-6,8a-dimethoxy-1,1a,2,8,8a,8b-hexahydro-5-methyl-arizino[2',3',3,4,]pyrrolo[1,2-a]indole-4,7-dione according to which the azirinopyrroloindole ring system is numbered as follows:



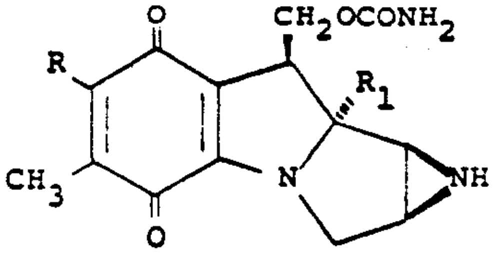
Chemical Abstracts

A trivial system of nomenclature which has found wide use in the mitomycin literature identifies the foregoing ring system including several of the characteristic substituents of the mitomycins as mitosane.



Mitosane

According to this system, mitomycin A is 7,9a-dimethoxymitosane and mitomycin C is 7-amino-9a-methoxymitosane. As to the stereochemical configuration of the products of this invention, it is intended when identifying them by the root name "mitosane" or by structural formula to identify the stereochemical configuration thereof as the same as that of mitomycin A or C.



Mitomycin A $R=R_1=OCH_3$
Mitomycin C $R=NH_2$, $R_1=OCH_3$

2. Disclosure Statement

Mitomycin C is an antibiotic which is produced by fermentation and is presently on sale under Food and Drug Administration approval in the therapy of disseminated adenocarcinoma of the stomach or pancreas in proven combinations with other approved chemotherapeutic agents and as palliative treatment when other modalities have failed (Mutamycin® Bristol Laboratories, Syracuse, New York 13221, Physicians' Desk Reference 37th Edition, 1983, pp. 747 and 748). Mitomycin C and its production by fermentation is the subject of U.S. Patent No. 3,660,578 patented May 2, 1972 claiming priority from earlier applications including an application filed in Japan on April 6, 1957.

The structures of mitomycins A, B, C, and of porfiromycin were first published by J. S. Webb et al. of Lederle Laboratories Division American Cyanamid Company, J. Am. Chem. Soc., 84, 3185-3187 (1962). One of the chemical transformations used in this structure study to relate mitomycin A and mitomycin C was the conversion of the former, 7,9a-dimethoxymitosane, by reaction with ammonia to the latter, 7-amino-9a-methoxymitosane. Displacement of the 7-methoxy group of mitomycin A has proven to be a reaction of considerable interest in the preparation of antitumor active derivatives of mitomycin C. Recently the stereochemical configurations of positions 1, 1a, 8a and 8b have been shown to be as indicated above with respect to the Chemical Abstracts nomenclature [Shirhata et al., J. Am. Chem. Soc., 105, 7199-7200 (1983)]. The earlier literature refers to the enantiomer.

The following articles and patents deal *inter alia* with the conversion of mitomycin A to a 7-substituted amino mitomycin C derivative having antitumor activity. The object of this research was to prepare derivatives which were more active, and particularly which were less toxic than mitomycin C:

Matsui *et al.*, *J. Antibiotics*, XXI, 189-198 (1968);

Konishita *et al.*, *J. Med. Chem.*, *14*, 103-109 (1971);

Iyengar *et al.*, *J. Med. Chem.*, *24*, 975-981 (1981);

Iyengar, Sami, Remers and Bradner, Abstracts of Papers, 183rd Annual Meeting of the American Chemical Society, Las Vegas, Nevada, March 1982, Abstract No. MEDI 72;

Cosulich *et al.*, U.S. Patent No. 3,332,944, issued July 25, 1967;

Matsui *et al.*, U.S. Patent No. 3,420,846, issued January 7, 1969;

Matsui *et al.*, U.S. Patent No. 3,450,705, issued June 17, 1969;

Matsui *et al.*, U.S. Patent No. 3,514,452, issued May 26, 1970;

Nakano *et al.*, U.S. Patent No. 4,231,936, issued November 4, 1980;

Remers, U.S. Patent No. 4,268,676, issued May 19, 1981.

The following patent applications deal with the preparation of 7-substituted amino mitomycin C derivatives in which the substituent incorporates a disulfide linkage.

Kono et al., European Patent Application No. 116,208 (1984);

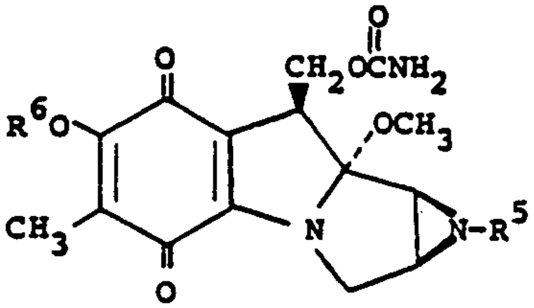
Vyas et al., U.K. Patent Application No. 2,140,799 (1984).

7-Alkoxy substituted mitosanes related structurally to mitomycin A are described as useful antibiotics having activity in experimental animal tumors in an article by Urakawa et al., J. Antibiotics, 23, 804-809 (1980).

Mitomycin C is the principal mitomycin produced by fermentation and is the commercially available form. Current technology for the conversion of mitomycin C to mitomycin A suffers from a number of deficiencies. Hydrolysis of mitomycin C to the corresponding 7-hydroxy-9a-methoxy-mitosane, and then methylation of that substance requires diazomethane, a very hazardous substance to handle on a manufacturing scale, and the 7-hydroxy intermediate is very unstable [Matsui et al., J. Antibiotics, XXI, 189-198 (1968)]. One attempt to avoid these difficulties involves the use of 7-acyloxymitosanes (Kyowa Hakko Kogyo KK Japanese Patent No. J5 6073-085, Farmdoc No. 56227 D/31). Alcoholysis of mitomycin A as described by Urakawa et al., J. Antibiotics, 23, 804-809 (1980) is limited to the production of only specific 7-alkoxy structural types by the availability and reactivity of the alcohol starting materials.

SUMMARY OF THE INVENTION

The present invention provides a process for preparing a compound having Formula IX

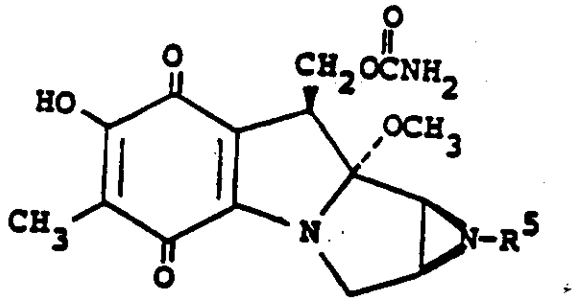


IX

wherein:

R^5 is hydrogen, or C_{1-6} alkyl, and

R^6 is C_{1-12} alkyl or substituted C_{1-12} alkyl, C_{3-12} cycloalkyl or substituted C_{3-12} cycloalkyl wherein the carbon atom thereof which is attached to the mitosane 7-oxygen atom bears from 1 to 2 hydrogen atoms and said substituents are selected from the group consisting of halogen, C_{1-6} alkoxy, C_{1-6} alkanoyl, C_{6-14} aroyl, cyano, trihalomethyl, amino, C_{1-6} monoalkylamino, C_{2-12} dialkylamino, C_{6-12} aryl, C_{6-12} aryloxy, C_{1-6} alkanoyloxy, C_{7-14} aroyloxy, heterocyclo having 1 or 2 rings and from 5 to 12 ring atoms including up to 4 heteroatoms selected from nitrogen, oxygen, and sulfur, and wherein each of said alkoxy, alkanoyl, aroyl, aryl, aryloxy, alkanoyloxy, aroyloxy, and heterocyclo substituents optionally contains from 1 to 2 substituents selected from halogen, C_{1-6} alkoxy, C_{1-6} alkanoyl, cyano, trihalomethyl, amino, C_{1-6} alkylamino, or C_{2-12} dialkylamino groups, provided that R^6 may also be 2-benzylthiolethyl or CH_2COOEt, which process comprises reacting a mitosane of Formula X



X

with a triazene of Formula XI

$$Ar\text{-}N{=}N\text{-}NH\text{-}R^6 \qquad XI$$

wherein R^5 and R^6 are as defined above and Ar is the organic residue of a diazotizable aromatic amine.

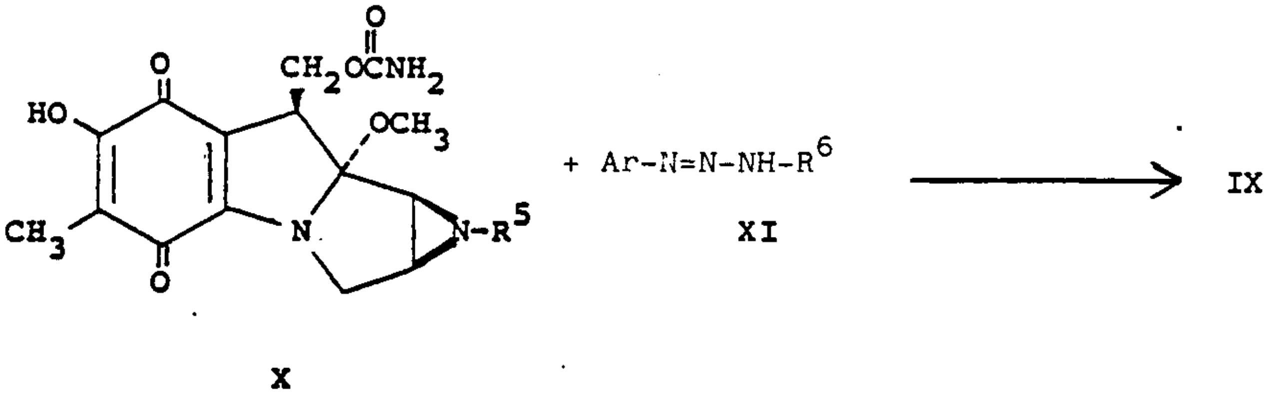
Many of the compounds of Formula IX are known compounds having inhibitory activity against experimental animal tumors in vivo. A number of novel compounds conforming to Formula IX have also been prepared by this process, and are considered part of the present invention. In particular the substances identified herein as compounds of Example Nos. 14, 15, 16 and 18 are novel substances, and also have antitumor activity against experimental animal tumors. These compounds are part of the present invention. They are employed in a manner similar to mitomycin C. The dosages employed are adjusted in proportion to their toxicities relative to the toxicity of mitomycin C. In cases where the new compound is less toxic, a higher dose is employed.

The terms "lower alkyl", "lower alkoxy" and "lower alkanoyl" as used herein and in the claims (unless the context indicates otherwise) mean straight or branched chain alkyl, alkoxy or alkanoyl groups containing from 1 to 6 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, amyl, hexyl, etc. Preferably, these groups contain from 1 to 4 carbon atoms and, most preferably, they contain 1 or 2 carbon atoms. Unless otherwise specified in the particular instance, the term "halogen" as used herein and in the claims is intended to include chlorine, fluorine, bromine and iodine. The term "nontoxic pharmaceutically acceptable salt" is intended to include salts of the compounds of Formulas I and II with any nontoxic pharmaceutically acceptable acid or base. Such acids are well-known and include hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, maleic, fumaric, succinic, oxalic, benzoic, methanesulfonic, tartaric, citric, camphorsulfonic, levulinic and the like. Such bases are well-known and include, e.g. nontoxic metallic salts such as sodium, potassium, calcium and magnesium, the ammonium salt and salts with nontoxic amines, e.g. trialkylamines, procaine, dibenzylamine, pyridine, N-methylmorpholine, N-methylpiperidine and the like. The salts are made by methods known in the art.

DESCRIPTION OF THE INVENTION

As stated above, the invention provides a new process for the preparation of compounds of Formula IX which comprises reacting a mitosane of Formula X with a triazene of Formula XI as shown in Scheme 1.

Scheme 1



wherein R^5 and R^6 are as defined above and Ar is the organic residue of a diazotizable aromatic amine.

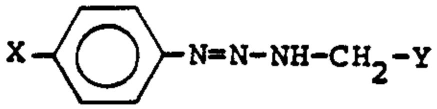
The 1-substituted-3-aryltriazenes of Formula XI and more specifically 1-alkyl-3-aryltriazenes make up a class of reagents which are known to be useful for reacting with carboxylic acids to form the corresponding lower alkyl esters. 1-Methyl-3-(4-methylphenyl)triazene may be prepared according to the general procedures described by E. H. White et al. in Org. Syn., 48, 102-105 (1968) and as described herein in Procedure 1. However, this procedure works well only with water-soluble amines, and a second procedure which is described by E. H. White et al., Tetrahedron Letters, No. 21, 761 (1961) and also described herein in Procedure 2 is more suitable for the preparation of triazenes of water-insoluble amines.

The reagent 1-methyl-3-(4-methylphenyl)triazene prepared in the above fashion has been previously employed to prepare methyl esters of carboxylic acids such as 2,4-dinitrobenzoic acid [E. H. White et al. Org. Syn., 48, 102-105 (1968)] and cephalosporanic acids which yields the desired Δ^3-compound without isomerization to the Δ^2-isomer [Mangia, Tetrahedron Letters, No. 52, pp. 5219-20 (1978)]. The reagent has also been employed to produce a 3-methoxy-cephalosporin derivative by reaction with the corresponding 3-hydroxy-3-cephem-4-carboxylate in benzene solution at the reflux temperature. (Wiederkeher et al. U.S. Patent No. 4,069,324 issued January 17, 1978).

Other 1-(lower alkyl)-3-aryltriazenes of Formula XI may be prepared similarly by reaction of other lower alkyl amines with aryldiazonium salts in similar fashion. Any arylamine having 6 to 12 carbon atoms which readily forms a diazonium salt may be used as the source of the aryl portion of the 1,3-disubstituted triazene. Some examples of triazenes produced in this fashion and used in the present invention are:

1-(n-butyl)-3-(4-methylphenyl)triazene;
1-(1-methylethyl)-3-(4-methylphenyl)triazene;
1-(4-methylphenyl)-3-[2-(4-morpholinyl)ethyl]triazene;
1-(4-methylphenyl)-3-[2-(2-pyridyl)ethyl]triazene;
1-(2-benzylthiolethyl)-3-(4-methylphenyl)triazene;
1-(4-chlorophenyl)-3-(2-methoxyethyl)triazene;
1-(4-chlorophenyl)-3-(1,3-dioxol-2-ylmethyl)triazene;
1-(4-chlorophenyl)-3-(tetrahydrofuran-2-ylmethyl)triazene.

Other triazenes have been described in the literature which are suitable reactants for use in the present process to provide 7-(substituted alkoxymitosane) of Formula IX. Those described by T. A. Daniels et al., Can. J. Chem., 55, 3751-3754 (1977) are exemplary.



a X = H, Y = CN
b X = NO_2, Y = CN
c X = CO_2Me, Y = CN
d X = Ac, Y = CN
e X = NO_2, Y = CO_2Et
f X = CO_2Me, Y = CO_2Et
g X = CO_2Me, Y = COPh
h X = NO_2, Y = $-CH(OCH_3)_2$

The following further exemplify suitable triazene starting materials of Formula XI for use in the present invention.

1-(n-butyl)-3-(α-napthyl)triazene
1-(n-hexyl)-3-phenyltriazene
1-ethyl-3-(2,4-dimethylphenyl)triazene
1-(1-methylethyl)-3-(4-methoxyphenyl)triazene

For the preparation of mitomycin A, we prefer to use 3-methyl-1-(4-methylphenyl)triazene as methylating reagent. Preferably, at least two molecular proportions of the latter per molecular proportion of 7-hydroxy-9a-methoxymitosane are employed and the reaction is preferably carried out in a liquid organic solvent for the 7-hydroxy-9a-methoxymitosane starting material. Preferred solvents are the lower alkanols, lower alkanoic lower alkyl esters, the dilower alkyl ethers, the cyclic aliphatic ethers, and the lower polyhalogenated aliphatic hydrocarbons. These solvents contain up to 6 carbon atoms, but those boiling at temperatures of less than 100°C are preferred. Specific preferred solvents are methylene chloride, methanol, diethyl ether, ethyl acetate, and mixtures thereof. The reaction may be carried out at the reflux temperature of the reaction mixture or up to about 60°C. At temperatures in excess of this the mitosane reactant is inclined to decompose with a resultant reduction in yield. It is preferred to carry out the reaction at room temperature or below, for instance within the range of 0 to 25°C.

A convenient way to determine when the reaction is complete is by thin layer chromatography. Mitomycin A is deep purple in color and can be readily distinguished from the starting material and from by-products. In the solvent system methylene chloride/methanol (90/10) mitomycin A exhibits R_f = 0.36. Chromatography on neutral alumina may be used for purification of the product.

The foregoing reaction conditions and precautions are generally applicable to the preparation of other 7-R^6O-mitosanes of Formula IX according to the present process.

Usefulness of compounds of Formula IX in antineoplastic therapeutic methods is demonstrated by the results of in vivo screening procedures wherein the compounds are administered in varying dosage amounts to mice in which a P-388 leukemic condition is induced.

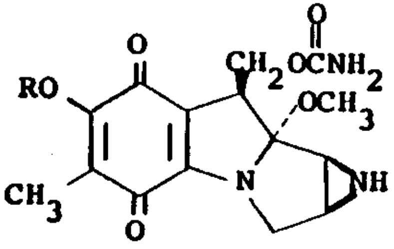
Compounds according to the present invention are believed to possess anti-bacterial activity against gram-positive and gram-negative microorganisms in a manner similar to that observed for the naturally occurring mitomycins and are thus potentially useful as therapeutic agents in treating bacterial infections in humans and animals.

Activity Against P-388 Murine Leukemia

Table I contains the results of laboratory tests with CDF_1 mice implanted intraperitoneally with a tumor inoculum of 10^6 ascites cells of P-388 murine leukemia and treated with various doses of either a test compound of Formula I or II, or with mitomycin C. The compounds were administered by intraperitoneal injection. Groups of six mice were used for each dosage amount and they were treated with a single dose of the compound on the day after inoculation. A group of ten saline treated control mice was included in each series of experiments. The mitomycin C treated groups were included as a positive control. A 30 day protocol was employed with the mean survival time in days being determined for each group of mice and the number of survivors at the end of the 30 day period being noted. The mice were weighed before treatment and again on day six. The change in weight was taken as a measure of drug toxicity. Mice weighing 20 grams each were employed and a loss in weight of up to approximately 2 grams was not considered excessive. The results were determined in terms of % T/C which is the ratio of the mean survival time of the treated group to the mean survival time of the saline treated control group times 100. The saline treated control animals usually died within nine days. The "maximum effect" in the following Table is expressed as % T/C and the dose giving that effect is given. The values in parenthesis are the values obtained with mitomycin C as the positive control in the same experiment. Thus a measure of the relative activity of the present substances to mitomycin C can be estimated. A minimum effect in terms of % T/C was considered to be 125. The minimum effective dose reported in the following Table is that dose giving a % T/C of approximately 125. The two values given in each instance in the "average weight change" column are respectively the average weight change per mouse at the maximum effective dose and at the minimum effective dose.

TABLE I

Inhibition of P-388 Murine Leukemia



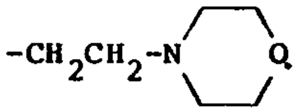
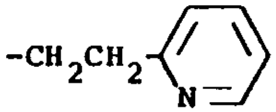
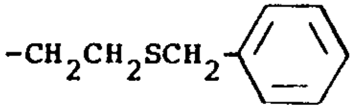
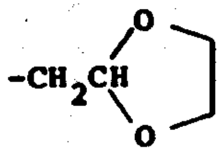
Compound of Example No.	R	Maximum Effect % T/C	 Dose[1]	Minimum Effect Dose[1]	Average Wt. Change[2]
12[3]	n-Bu	167(306)[4]	1.6(3.2)[4]	0.2	+0.1; -0.4
13[3]	i-Pr	167(306)	1.6(3.2)	0.05	-0.7; +0.1
14		222(306)	6.4(3.2)	<0.05	-1.7; -0.5
15		233(306)	3.2(3.2)	<0.05	-2.4; +0.2
16		156(306)	6.4(3.2)	0.2	-0.7; +0.3

TABLE I (continued)

Compound of Example No.	R	Maximum Effect		Minimum Effect	Average
		% T/C	Dose[1]	Dose[1]	Wt. Change[2]
17[3]	$-CH_2CH_2OCH_3$	211(172)	0.4(3.2)	<0.05	-0.6; +1.3
18		267(172)	0.4(3.2)	<0.05	+0.9; +1.7

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1. mg/kg of body weight
2. average grams per day for each maximal and minimal effective doses
3. Urakawa et al., J. Antibiotics, 23, 804-809 (1980)
4. values in parentheses are for mitomycin C tested in the same run

In view of the antitumor activity observed in experimental animal tumors, we contemplate the use of the substances of the present invention for inhibiting mammalian tumors. For this purpose, they are administered systematically to a mammal bearing a tumor in substantially nontoxic antitumor effective dose.

The compounds of the present invention are intended primarily for use by injection in much the same way and for some of the same purposes as mitomycin C. Somewhat larger or smaller doses may be employed depending upon the particular tumor sensitivity. They are readily distributed as dry pharmaceutical compositions containing diluents, buffers, stabilizers, solubilizers and ingredients contributing to pharmaceutical elegance. These compositions are then constituted with an injectable liquid medium extemporaneously just prior to use. Suitable injectable liquids include water, isotonic saline and the like.

DESCRIPTION OF SPECIFIC EMBODIMENTS

In the following procedures and examples, all temperatures are given in degrees Centigrade, and melting points are uncorrected. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian*XL100, Joel FX-90Q or Bruker WM 360 spectrometer in either pyridine-$\underline{d}_5$ or D_2O as indicated. When pyridine-$\underline{d}_5$ is used as the solvent, the pyridine resonance at δ=8.57 is used as an internal reference, whereas with D_2O as solvent TSP is used as the internal reference. Chemical shifts are reported in δ units and coupling constants in Hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad signal; dd, doublet of doublet; dt, doublet of triplet. Infrared spectra were determined either on a Beckman*Model 4240 spectrometer or a Nicolet 5DX FT-IR spectrometer and are reported in reciprocal centimeters. Ultraviolet (UV) spectra were determined either on a Cary Model 290 spectrometer or a Hewlitt Packard 8450A spectrometer equipped with a multidiode array

* **Registered Trade Mark**

detector. Thin layer chromatography (TLC) was carried out on 0.25mm Analtech silica gel GF plates. Flash chromatography was run with either Woelm neutral alumina (DCC grade) or Woelm silica gel (32-63μm) and the indicated solvents. All evaporations of solvents were performed under reduced pressure and below 40°C.

The 1-alkyl-3-aryltriazenes make up a class of reagents which are known to be useful for reacting with carboxylic acids to form the corresponding lower alkyl esters. 1-Methyl-3-(4-methylphenyl)triazene may be prepared as follows:

Procedure 1 E. H. White et al., Org. Syn., 48, 102-195 (1968).

1-Methyl-3-p-tolyltriazene. p-Toluidine (50.2 g, 0.47 mole) is added to a 2-l flask equipped with a 200-ml dropping funnel and an efficient stirrer, and the flask is immersed in an ice-salt bath at ca. -10°. A solution of 46.8 g (0.55 mole) of potassium nitrite in 150 ml of water is placed in the dropping funnel, and a mixture of 250 g of crushed ice and 140 ml of concentrated hydrochloric acid is added to the p-toluidine with stirring. The potassium nitrite solution is slowly added with continued stirring during 1-2 hours until a positive starch-potassium iodide test is obtained (Note 1), and the mixture is stirred for an additional hour to ensure the reaction of all the toluidine.

The solution of p-toluenediazonium chloride is then brought to pH 6.8-7.2 at 0° with cold, concentrated, aqueous sodium carbonate, whereupon the solution becomes red to orange in color and a small amount of red material settles out. The cold, neutral solution is transferred to a dropping funnel and added slowly to a vigorously stirred mixture of 150 g of sodium carbonate, 300 ml of 30-35% aqueous methylamine (Note 2), and 100 g of crushed ice in a 3-l flask. The reaction mixture is kept at ca. -10° during the addition, which requires about 45 minutes (Note 3). The solution is extracted with three 1-l portions of ether. The ethereal extracts are dried with anhydrous sodium sulfate and evaporated on a rotary evaporator at room temperature to give 65 g of crude 1-methyl-3-p-tolyltriazene (Note 4). This is placed in a water-cooled sublimer, and the triazene is sublimed at 50° (1 mm.); 43.3 g (0.29 mole, 62%) of a yellow, crystalline sublimate, m.p. 77-80°, is obtained (Note 5). The sublimate can be recrystallized from hexane to give the triazene as white needles, m.p. 80.5-81.5°. More conveniently, it is dissolved in the minimum amount of ether, and the solution is diluted with 2 volumes of hexane and cooled to 0° to give flat plates with a slightly yellow cast; m.p. 79-81°. The yield of pure triazene is 33-37 g (47-53%) (Note 6).

NOTES

1. The individual tests with starch-potassium iodide paper should be made 1-2 minutes after the addition of potassium nitrite has been stopped.

2. 40% aqueous methylamine may be substituted.

3. The reaction is over when a drop of solution no longer gives a red color with a solution of β-naphthol in aqueous sodium carbonate.

4. The chief impurity is 1,5-di-p-tolyl-3-methyl-1,4,-pentazadiene (m.p. 148°). This can be removed by fractional crystallization, but it is easier to sublime the triazene from the reaction mixture.

5. The sublimate contains a trace of 1,3-di-p-tolyltriazene, as shown by thin-layer chromatography. Recrystallization yields the pure 1-methyl-3-p-tolyltriazene.

6. This procedure works well only with water-soluble amines. Procedure 2 given below is more suitable for the preparation of triazenes of water-insoluble amines.

Procedure 2 E. H. White et al., Tetrahederon Letters No. 21, p. 761 (1961).

1-n-Butyl-3-p-chlorophenyltriazene. A solution of p-chlorobenzenediazonium hexafluorophosphate (recrystallized from acetone-methanol) (2.87 g, 10.1 mmoles) in dimethylformamide (dimethylamine-free) was added slowly to a stirred mixture of n-butylamine (0.73 g, 10.0 mmoles), powdered sodium carbonate (15 g), and dimethylformamide (30 ml) stirred and maintained at -5°. The diazonium salt solution may be used at room temperature; however, a purer product is usually obtained if the diazonium salt solution is prepared in and delivered from a cooled separatory funnel maintained at ca. -50°. The mixture was warmed to 0° and stirred until a negative test was obtained with 2-naphthol (only a few minutes are usually required). Ether was added, the mixture was filtered, and the filtrate was washed thoroughly with water, then dried. (The triazene may be isolated at this point and recrystallized from pentane at low temperatures.

Procedure 3

7-Hydroxy-9a-methoxymitosane. Mitomycin C (2.2 g, 6.6 mmoles) was dissolved in 140 ml 0.1N methanolic NaOH (50%) and the reaction mixture was stirred at room temperature for 30 hours. The solution was then adjusted to ca. pH 4.0 with 1N HCl and extracted with ethyl acetate (4 x 500 ml). The combined ethyl acetate extracts were dried (Na_2SO_4) and evaporated under reduced pressure at about 30 to 35°C to obtain a solid residue, which upon dissolving in ether and treating with excess hexane afforded a purple precipitate. The precipitate was collected and air dried to afford the title compound as a fine purple powder (1.4 g, 63%).

^1H NMR (pyridine-$\underline{d}_5$, δ): 2.05(s, 3H), 2.14(bs, 1H), 2.74(bs, 1H), 3.13(d, 1H), 3.24(s, 3H), 3.56(d, 1H), 4.00(dd, 1H), 4.37(d, 1H), 5.05(t, 1H), 5.40(dd, 1H), 5.90(bs, 2H).

Procedure 4

Mitomycin A. A 100 mg (0.30 mmole) quantity of 7-hydroxy9a-methoxymitosane and 100 mg (0.67 mmole) quantity of 3-methyl-1-p-tolyltriazene was dissolved in 2 ml methylene chloride and 10 ml diethyl ether. The solution, after gently refluxing for 6 hours was stirred at room temperature for 18 hours. TLC [methylene chloride:methanol (90:10)] revealed the appearance of a deep purple spot at R_f=0.36 with a trace amount of impurity at R_f=0.41. The reaction mixture was concentrated to dryness and chromatographed on Woelm neutral alumina employing methylene chloride and methylene chloride:methanol (30:1) as eluting solvents. Fractions containing the component at R_f=0.36 were pooled and concentrated to dryness. Precipitation of the dry residue from methylene chloride and hexane afforded the title compound as a fine amorphous purple powder (25 mg, 24%), mp 161°.

Anal. Calc'd for $C_{16}H_{19}N_3O_6$: C, 54.96; H, 5.44; N, 12.02
Found: C, 53.96; H, 5.37; N, 11.99

IR(KBr), ν_{max}, cm^{-1}: 3400, 3300, 2950, 1700, 1630, 1575, 1200, 1060.

^1H NMR (pyridine-d_5, δ): 1.82(s, 3H), 2.74(dd, 1H), 3.12(d, 1H), 3.24(s, 3H), 3.54(dd, 1H), 3.96(dd, 1H), 4.02(s, 3H), 4.22(d, 1H), 4.84(bs, 2H), 5.02(t, 1H), 5.38(dd, 1H).

The yield in procedure 4 is raised to 63% by employing methylene chloride as reaction solvent and room temperature for a 24 hour period.

Procedure 5

In a 250 ml one neck round bottom flask was placed solid Na_2CO_3, 35% aqueous solution of amine (amount as in Procedure 1) and ice, and the suspension was stirred at -5°C (ice-salt bath). To this suspension was added dropwise, a cold suspension of p-chlorobenzenediazonium hexafluorophosphate

(Aldrich Chemical Co.) in ice, water, Na_2CO_3 (solution about pH 7). After the addition was complete, the reaction mixture was extracted with diethyl ether. The combined diethyl ether extract was backwashed with water, dried (Na_2SO_4) and concentrated. The yellowish solid residue was purified by column chromatography over Woelm alumina using hexane-methylene chloride (1:1) as eluting solvent (^{1}H NMR recorded).

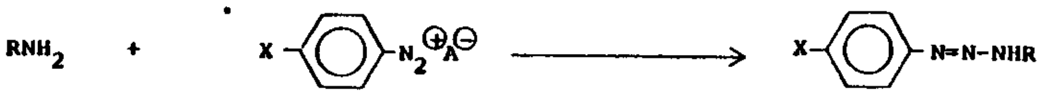
Examples 1-10

The triazenes 1-7 of Table II which follows were prepared according to general Procedure 1 described above wherein the triazene of Example 1 is exemplified. The triazenes were purified by column chromatography on Woelm alumina.

The triazenes 8-10 of Table II were prepared according to general Procedure 5 described above.

TABLE II

Triazenes



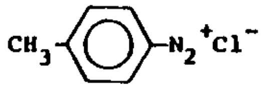
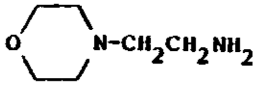
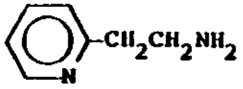
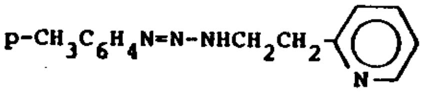
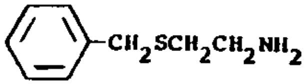
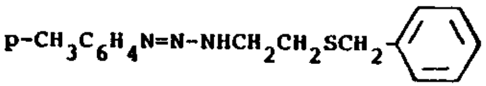
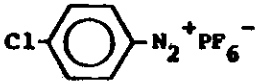
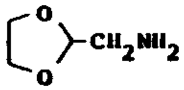
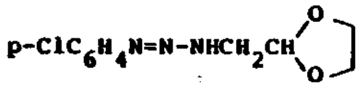
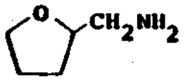
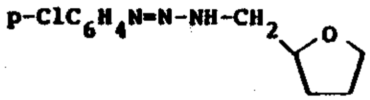
Example No.	Amine	Diazonium Salt	Triazene
1	CH_3NH_2		$p\text{-}CH_3C_6H_4N{=}N\text{-}NHCH_3$
2	$CH_3(CH_2)_3NH_2$	"	$p\text{-}CH_3C_6H_4N{=}N\text{-}\overset{H}{N}(CH_2)_3CH_3$
3	$(CH_3)_2CHNH_2$	"	$p\text{-}CH_3C_6H_4N{=}N\text{-}NHCH(CH_3)_2$
4		"	
5		"	
6		"	
7	$CH_3OCNHCH_2CH_2SSCH_2CH_2NH_2$	"	$p\text{-}CH_3C_6H_4N{=}N\text{-}NHCH_2CH_2SSCH_2CH_2NHCOCH_3$

TABLE II (continued)

Example No.	Amine	Diazonium Salt	Triazene
8	$CH_3OCH_2CH_2NH_2$		p-$ClC_6H_4N{=}N{-}NHCH_2CH_2OCH_3$
9		"	
10		"	

Example 11

1-[2-(3-Nitro-2-pyridyldithio)ethyl]-3-(4-methylphenyl)-triazene

A solution of 4-methylphenyldiazonium chloride is prepared as described in Procedure 1 from *p*-toluidine and adjusted to pH 6.8-7.2 at 0°C as described in that procedure. A solution containing 21.15 mmoles of the diazonium salt in 45 ml of solution was prepared in this fashion and placed in a dropping funnel connected to a 250 ml 3 neck, round bottom flask containing 5.34 g (20.0 mmoles) of 2-(3-nitro-2-pyridyldithio) ethylamine, 7 g of sodium carbonate, and 150 ml of dioxane which had been added to the flask in that sequence. Saturated aqueous sodium carbonate solution, 6 ml, and 10 g of ice were added to the flask. The flask was chilled in an ice bath and the contents stirred mechanically. The diazonium salt solution was then added dropwise during a one hour period from the dropping funnel. When addition had been completed the reaction mixture was allowed to warm to room temperature and was then extracted with three 400 ml portions of ether. Drying and evaporation of the extracts yielded the desired product which was purified by chromatography using an alumina packed column, one inch in diameter and ten inches long, using hexane:methylene chloride (4:1); hexane:methylene chloride (3:2); hexane:methylene chloride (1:4); and finally methylene chloride containing 1% methanol for development and elution of the column. The appropriate fractions (identified by TLC) were combined and evaporated to yield 2.5 g of the title compound.

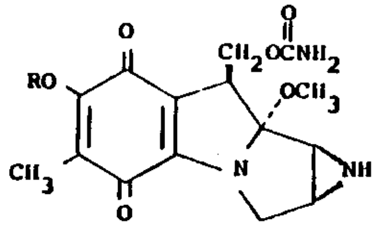
Examples 12-18

General procedure for preparing 7-alkoxy-9a-methoxymitosanes (12-18)

A solution of triazene (2.4 equivalents) in CH_2Cl_2:methanol (4:1) was added to a solution of 7-hydroxy-9amethoxymitosane (prepared in Procedure 3) in CH_2Cl_2:methanol (4:1). The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by thin layer chromatography (TLC) (10% MeOH in CH_2Cl_2). The 7-alkoxy-9a-methoxymitosane product appears as a dark purple spot on the TLC. The reaction mixture is chromatographed on Woelm alumina when the reaction is judged to be complete on the basis of the TLC, and the 7-alkoxy-9a-methoxymitosane is obtained as an amorphous solid. The products produced are identified as Example Nos. 12-18 in Table III.

TABLE III

7-Alkoxy-9a-methoxymitosanes



Example No.	Formula	^{1}H NMR (Pyridine-$\underline{d}_5$, δ) ppm	IR (cm^{-1})	Elemental Analysis[a]
12[b]	R=-$CH_2CH_2CH_2CH_3$	0.80(t, 3H), 1.44(m, 4H), 1.84(s, 3H), 2.08(bs, 1H), 2.72(bs, 1H), 3.08(bs, 1H), 3.20(s, 3H), 3.52(d, 1H), 4.00(dd, 1H), 4.28(m, 3H), 5.04(t, 1H), 5.44(dd, 1H), 7.64(bs, 2H).	-	T: C, 58.30; H, 6.44; N, 10.74 F: C, 58.34; H, 6.25; N, 10.80
13[b]	R=-$CH(CH_3)_2$	1.20(d, 3H), 1.28(d, 3H), 1.88(s, 3H), 2.20(bs, 1H), 2.76(bs, 1H), 3.16(bs, 1H), 3.24(s, 3H), 3.56(d, 1H), 4.00(dd, 1H), 4.24(d, 1H), 5.00(m, 2H), 5.44(dd, 1H).	-	-

TABLE III (continued)

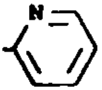
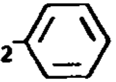
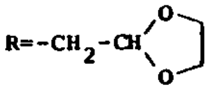
Example No.	Formula	^{1}H NMR (Pyridine-d_5, δ) ppm	IR (cm^{-1})	Elemental Analysis^a
14^{c,d}	R=-CH$_2$CH$_2$- 	1.96(s, 3H), 2.40(m, 4H), 2.56(t, 2H), 2.76(bs, 1H), 3.16(d, 1H), 3.24 (s, 3H), 3.54(d, 1H), 3.68(m, 4H), 4.00(dd, 1H), 4.26(d, 1H), 4.60(m, 2H), 5.04(t, 1H), 5.44(dd, 1H).	3480, 3260, 2940, 1725, 1620, 1210 1055	T: C, 52.60; H, 5.95; N, 11.41 F: C, 52.75; H, 6.00; N, 11.44 (Corrected for 0.5 mole % of CH$_2$Cl$_2$).
15	R=-CH$_2$CH$_2$- 	1.68(s, 3H), 1.96(bs, 1H), 2.72(bs, 1H), 3.08(bs, 1H), 3.20(t, 2H), 3.20(t, 3H), 3.50(d, 1H), 3.92(dd, 1H), 4.16(d, 1H), 4.92(m), 5.36(dd, 1H).	3430, 3300, 2930, 1715, 1625, 1210, 1060	T: C, 59.99, H, 5.49; N, 12.72 F: C, 59.94; H, 5.66; N, 12.63
16	R=-CH$_2$CH$_2$SCH$_2$- 	1.92(s, 3H), 2.12(bs, 1H), 2.76(bs, 1H), 2.84(t, 2H), 3.16(bs, 1H), 3.26(s, 3H), 3.56(d, 1H), 3.86(s, 2H), 4.04(dd, 1H), 4.28(d, 1H), 4.60(m, 2H), 5.12(t, 1H), 5.48(dd, 1H), 7.46(m), 7.76(bs).	3420, 3280, 2930, 1895, 1620, 1210, 1065	T: C, 59.37; H, 5.60; N, 8.65 S, 6.60 F: C, 59.26; H, 5.66; N, 8.63, S, 6.66

TABLE III (continued)

Example No.	Formula	^{1}H NMR (Pyridine-d_5, δ) ppm	IR (cm^{-1})	Elemental Analysis[a]
17[b]	R=-$CH_2CH_2OCH_3$	1.88(s, 3H), 2.00(bs, 1H), 2.72(bs, 1H), 3.08(d, 1H), 3.20(s, 3H), 3.24(s, 3H), 3.52(m, 3H), 3.96(dd, 1H), 4.20(d, 1H), 4.56(m, 2H), 5.04(t, 1H), 5.40(dd, 1H).	3440, 3280, 2950, 1700, 1630, 1200, 1065	T: C, 54.96; H, 5.89; N, 10.68 F: C, 54.45; H, 5.85; N, 10.42
18		1.92(s, 3H), 2.72(m, 1H), 3.08(d, 1H), 3.20(s, 3H), 3.52(dd, 1H), 3.84(m, 4H), 3.94(dd, 1H), 4.20(d, 1H), 4.60(m, 2H), 4.96(t, 1H), 5.28(m, 1H), 5.36(dd, 1H), 7.52(bs, 2H).	3460, 3360, 3200, 2960, 1720, 1625, 1210, 1060	T: C, 54.15; H, 5.50; N, 9.97 F: C, 53.44; H, 5.46; N, 9.74

a) T = theory, F = found

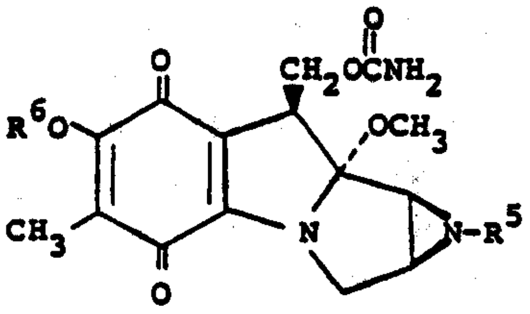
b) Urakawa *et al.*, *J. Antibiotics*, 23, 804-809 (1980)

c) Molecular ion observed in (+) and (-) CIMS

d) Also preparable by alcoholysis of mitomycin A, Urakawa *et al.*, *loc. cit.*

CLAIMS

1. The process for preparing a compound having Formula IX

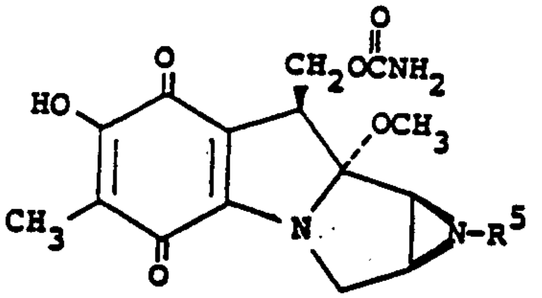


IX

wherein:

R^5 is hydrogen, or C_{1-6} alkyl, and

R^6 is C_{1-12} alkyl or substituted C_{1-12} alkyl, C_{3-12} cycloalkyl or substituted C_{3-12} cycloalkyl wherein the carbon atom thereof which is attached to the mitosane 7-oxygen atom bears from 1 to 2 hydrogen atoms and said substituents are selected from the group consisting of halogen, C_{1-6} alkoxy, C_{1-6} alkanoyl, C_{6-14} aroyl, cyano, trihalomethyl, amino, C_{1-6} monoalkylamino, C_{2-12} dialkylamino, C_{6-12} aryl, C_{6-12} aryloxy, C_{1-6} alkanoyloxy, C_{7-14} aroyloxy, heterocyclo having 1 or 2 rings and from 5 to 12 ring atoms including up to 4 heteroatoms selected from nitrogen, oxygen, and sulfur, and wherein each of said alkoxy, alkanoyl, aroyl, aryl, aryloxy, alkanoyloxy, aroyloxy, and heterocyclo substituents optionally contains from 1 to 2 substituents selected from halogen, C_{1-6} alkoxy, C_{1-6} alkanoyl, cyano, trihalomethyl, amino, C_{1-6} alkylamino, or C_{2-12} dialkylamino groups, provided that R^6 may also be 2-benzylthiolethyl or CH_2COOEt, which process comprises reacting a mitosane of Formula X



X

with a triazene of Formula XI

$$Ar-N=N-NH-R^6 \qquad XI$$

wherein R^5 and R^6 are as defined above and Ar is the organic residue of a diazotizable aromatic amine.

2. The process of Claim 1 wherein said triazene of Formula XI is 3-methyl-1-(4-methylphenyl)triazene.

3. The process of Claim 1 or 2 wherein at least two molecular proportions of said triazene relative to said mitosane of Formula X are employed.

4. The process of Claim 1, 2 or 3 wherein a reaction inert organic liquid solvent for said mitosane of Formula X is employed as reaction medium.

5. The process of Claim 1, 2 or 3 wherein a lower alkanol, a lower alkyl alkanoic ester, a dilower alkyl ether, a lower polyhalogenated aliphatic hydrocarbon or a cyclic aliphatic ether having up to 8 carbon atoms is employed as reaction medium.

6. The process of Claim 1, 2 or 3 wherein methylene chloride, methanol, diethyl ether, ethyl acetate or a mixture of two or more thereof is employed as reaction medium.

7. The process of any of claims 1 to 6 wherein the reaction temperature is from 0° to 60°C.

8. The process of Claim 7 wherein the reaction temperature is from 0° to 25°C.

9. The process of Claim 1 wherein R^6 is 2-(benzylthio)-ethyl and R^5 is hydrogen.

10. The process of Claim 1 wherein R^6 is 2-(2-pyridyl)-ethyl and R^5 is hydrogen.

11. The process of Claim 1 wherein R^6 is 2-(4-morpholinyl)ethyl and R^5 is hydrogen.

12. The process of Claim 1 wherein R^6 is (1,3-dioxolan-2-yl)methyl and R^5 is hydrogen.

13. A process as claimed in claim 1, substantially as described in respect of the foregoing Procedure 4 or any of the foregoing Examples 12 to 18.

14. A compound of the formula IX, as defined in claim 1, prepared by a process as claimed in any of claims 1 to 13.

15. The compound of the formula IX, as defined in claim 1, identified in respect of Example 14, 15, 16 or 18.

16. A pharmaceutical composition comprising a compound as claimed in claim 14 or 15 and a pharmaceutically acceptable solvent, diluent, adjuvant or carrier.

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Form 1 Application No GB8726516.1 filing date 03.09.1985

Lodged on 12.11.1987

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17.06.1985 in United States of America - doc: 744570

Earlier Application Under Section 15(4): GB8521816.2 Pubn. No GB2164038 filed on 03.09.1985

Title PREPARATION OF PYRROLOINDOLE DERIVATIVES

Applicant/Proprietor
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Entry Type 10.1 Staff ID. DG Auth ID. EO

10.01.1991 BRISTOL-MYERS SQUIBB COMPANY, Incorporated in USA - Delaware, 345 Park Avenue, New York, N.Y. 10154, United States of America [ADP No. 04454989001]

New

registered as Applicant/Proprietor in place of BRISTOL-MYERS COMPANY, Incorporated in USA - Delaware, 345 Park Avenue, New York, New York 10154, United States of America [ADP No. 00348961003] by virtue of merger dated 04.10.1989. Certified copy filed on GB2196006
Entry Type 8.4 Staff ID. DG Auth ID. EO

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        **** END OF REGISTER ENTRY ****
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RENEWAL DETAILS

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