15-(14′-Eburnamyl)pleiocarpinine (Pleiomutine). A New Dimeric Indole Alkaloid from *Pleiocarpa mutica* Benth

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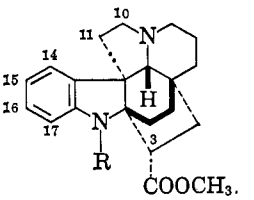
Abstract: A new dimeric indole alkaloid was isolated from the stem bark of *Pleiocarpa mutica* Benth. Mass spectra of the dimeric alkaloid and its cleavage products revealed it to be an eburnamylpleiocarpinine. The positions of attachment of the two monomeric units were deduced from nmr spectra. The proposed structure X was confirmed by partial synthesis.

In continuation of the study[2] of minor alkaloids from *Pleiocarpa mutica* Benth, we have isolated a new dimeric indole alkaloid. This compound, mp 225° dec, was obtained by repeated chromatography of the stem bark extract on both alumina and silicic acid.[2b] Its mass spectrum which is discussed in detail below indicated a molecular weight of 630, suggesting that the new compound belonged to the class of "dimeric" alkaloids.

An infrared spectrum of the unknown alkaloid had a single peak in the carbonyl region (1725 cm^{-1}) but none in the N–H and O–H region. The presence of a carbomethoxy group was confirmed by the nmr spectrum of the compound (3.8 ppm), as well as by reduction to a compound of mol wt 602 with lithium aluminum hydride (mol wt 604 with lithium aluminum deuteride). The mass spectrum of this reduction product was consistent with the conversion of a methyl ester to the corresponding primary alcohol. The nmr spectrum also disclosed the presence of an N-methyl group (2.8 ppm) and provided additional structural information which will be discussed in detail later.

The ultraviolet spectrum had no resemblance to spectra of other alkaloids of known structure; yet it was nearly identical with the spectrum of pleiomutine, a compound of undetermined structure isolated by Kump and Schmid[3] from *P. mutica*. These authors had already considered that the spectrum resulted from the combination of indole and indoline chromophores. Closer examination revealed that the ultraviolet spectrum can be approximated by superimposing the absorption of pleiocarpinine and an indole chromophore, moieties which were suggested by the mass spectrum (see below). Available data[4] for the amorphous pleiomutine are comparable with data for our alkaloid, which has the elemental composition of $C_{41}H_{50}N_4O_2$, determined by high-resolution mass spectrometry (calcd 630.3934, found 630.3915). Thus it seemed likely that our compound was pleiomutine. Their identity was subsequently confirmed by a direct comparison (R_f, infrared).

The mass spectrum of the new alkaloid (Figure 1) shows two very intense peaks at *m/e* 109 and 124. These peaks are characteristic[5] of several other alkaloids also found in *P. mutica*: pleiocarpinine (I), pleiocarpine (II), and kopsinine (III).[6] The occurrence of these peaks provided the first clue that one of the pleiocarpine-type alkaloids was present as the indoline moiety of the "dimer." More specifically, the presence of one N-methyl and one carbomethoxy group pointed to pleiocarpinine (I).



I, R = CH_3
II, R = $COOCH_3$
III, R = H

While the mass spectra of a large number of alkaloids with this carbon skeleton (although not pleiocarpinine itself) have previously been discussed in some detail,[5] it is considered important to present and discuss the spectrum of pleiocarpinine here (Figure 2) in order to facilitate direct comparison with the spectrum of the dimeric alkaloid.

Ion a arises by loss of the ethylene bridge, with a rearrangement characteristic of this carbon skeleton. Although a similar loss of the substituted bridge has not been observed in other compounds studied, the small peak at *m/e* 266 in the pleiocarpinine mass spectrum may well originate by this process. Cleavage of the two bonds indicated by dotted lines in structure a produces ion z, *m/e* 109. The formation of fragments c, d, and *m/e* 265 are self-explanatory as diagramed. (In the fragmentation schemes diagramed, all structures are ions, but positive charges are indicated only for even-electron ions. The charge is omitted from "molecular ions" rather than using awkward brackets around each structure or arbitrarily localizing the charge.) Rearrangement of a as illustrated leads to i, which subsequently loses the carbomethoxy group to form an ion of mass 170. Fragment k will be referred to later.

A second mode of rearrangement of the molecular ion involves[5] transfer of the C-3 proton to the ethylene

(1) National Institutes of Health Predoctoral Fellow, 1963–1966.

(2) (a) H. Achenbach and K. Biemann, *Tetrahedron Letters*, 3239 (1965); (b) *J. Am. Chem. Soc.*, **87**, 4177 (1965); (c) *ibid.*, **87**, 4944 (1965).

(3) W. G. Kump and H. Schmid, *Helv. Chim. Acta*, **44**, 1503 (1961).

(4) Pleiomutine[3]: $C_{42-43}H_{52-54}N_4O_2$ γ_{max} 1730 cm^{-1}, λ_{max}^{EtOH} 210 mμ (log ε 4.65), 233 (4.53), 264 (4.21), 286 (3.99), 294 (4.00); $[\alpha]^{22}D$ −97 ± 5° (*c* 1.03, $CHCl_3$); one OCH_3, one NCH_3.

(5) C. Djerassi, T. George, N. Finch, H. F. Lodish, H. Budzikiewicz, and B. Gilbert, *J. Am. Chem. Soc.*, **84**, 1499 (1962); C. Djerassi, H. Budzikiewicz, R. J. Owellen, J. M. Wilson, W. G. Kump, D. J. LeCount, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **46**, 742 (1963).

(6) W. G. Kump, D. J. LeCount, A. R. Battersby, and H. Schmid, *ibid.*, **45**, 854 (1962).

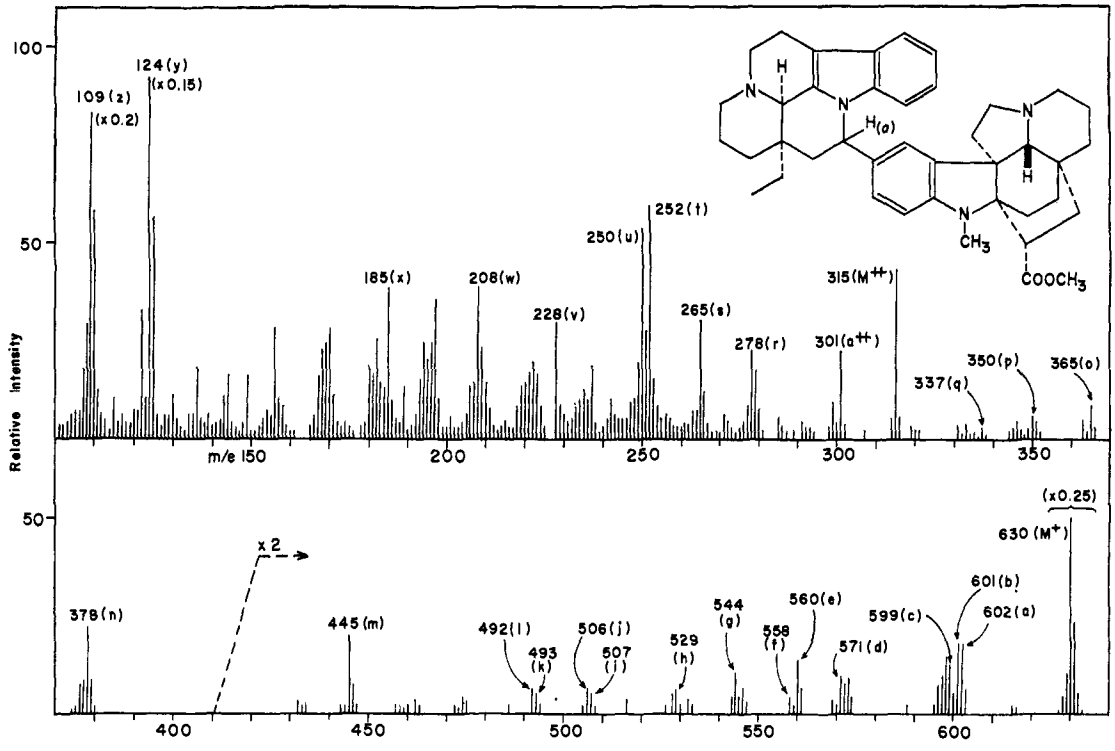
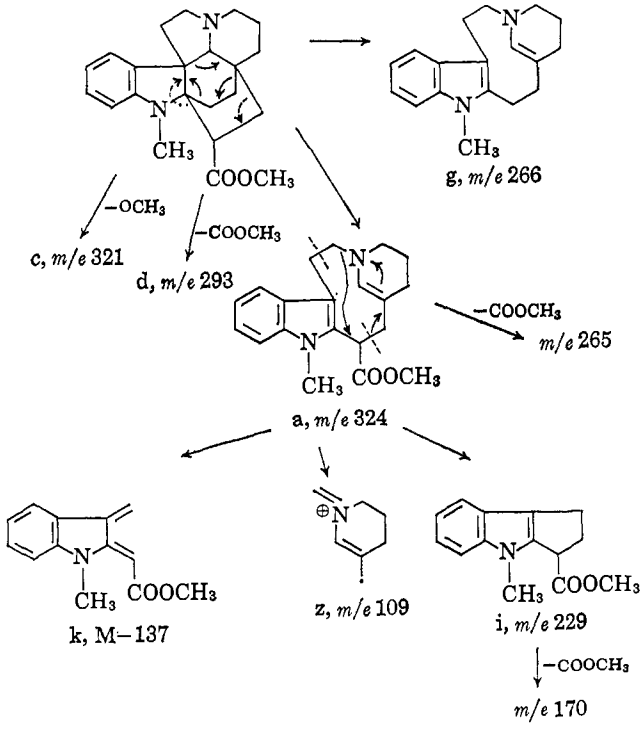


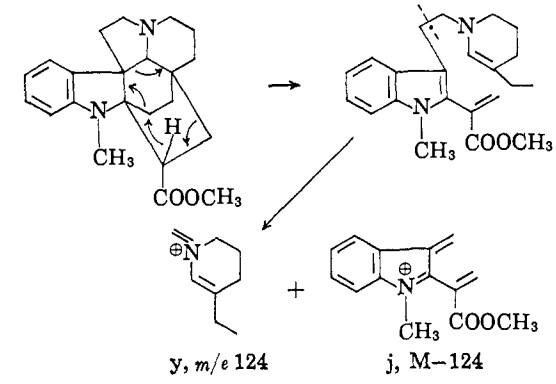
Figure 1. Mass spectrum of 15-(14′-eburnamyl)pleiocarpinine (X).

bridge. Cleavage then produces the abundant ion y, of mass 124. The accompanying fragment j, M − 124,

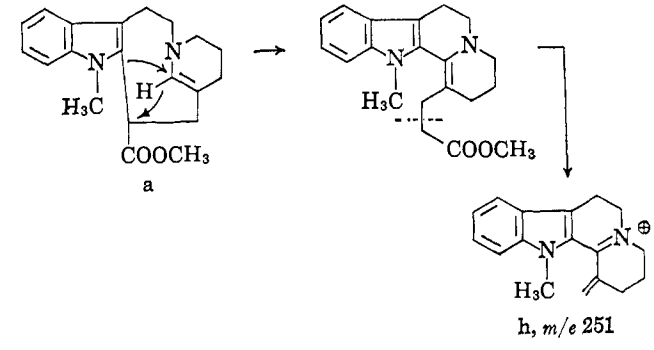


is not found to an appreciable extent in the spectrum of pleiocarpinine, but it is formulated here for later reference.

The origin of fragment h, *m/e* 251, is not immediately obvious. Related fragments appear at M − 101 in the spectrum of pleiocarpine (II), (M − $C_5H_9O_2$; detd 295.1421, calcd 295.1446), and at M − 73 for N-methylkopsinyl alcohol. Thus the substituent at C-3 is lost, together with three carbon atoms of the alicyclic



portion of the molecule. The following scheme is suggested for the formation of this ion.



An examination of the mass spectrum of the dimeric alkaloid (Figure 1) reveals fragments equivalent to nearly all major ions in the pleiocarpinine spectrum; y and z occur at the same masses, whereas a, c, d, g, h, and i are shifted 278 mass units, and therefore contain a substituent of mass 279. Consideration of the proposed structures for these ions requires that the second moiety must be attached either to C-11 or at the aromatic

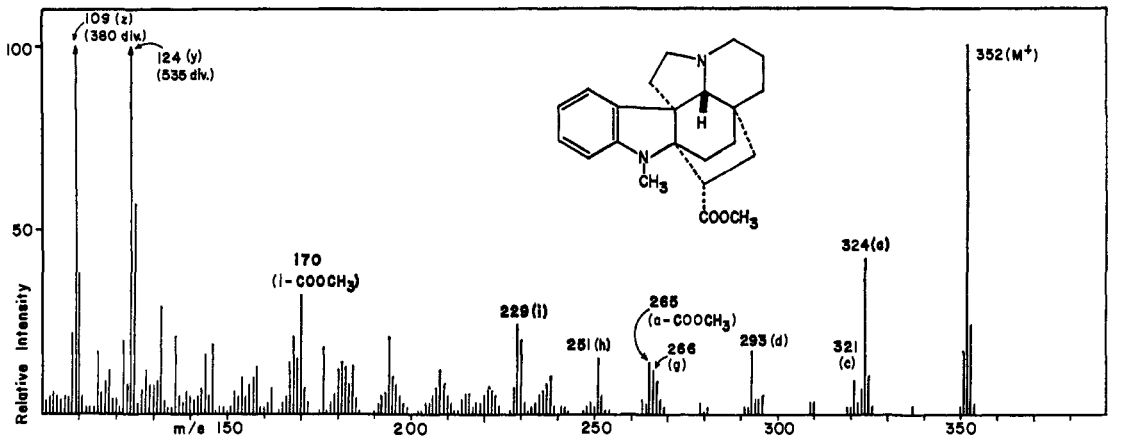
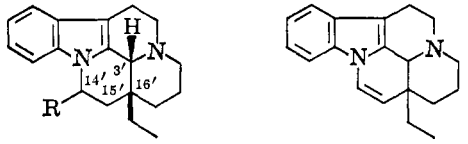


Figure 2. Mass spectrum of pleiocarpinine (I).

nucleus. Attachment at C-3 may be ruled out as a proton at the position is necessary to form fragment y, and the presence of an unmodified N-methyl group is required by the nmr spectrum of the alkaloid.

Upon subtraction of the elemental composition of pleiocarpinine minus a hydrogen from that of the "dimeric" alkaloid, a composition of $C_{19}H_{23}N_2$ was deduced for the as yet unknown moiety which replaces a hydrogen in the pleiocarpinine portion. A triplet at 0.9 ppm in the nmr spectrum is appropriate for the methyl signal of an ethyl substituent, the presence of which was also suggested by the significant mass spectral peak b at M − 29. A C_{19} skeleton with an ethyl group is characteristic of some eburnamine-type alkaloids,[7] at least one of which (eburnamenine (VI)) is a



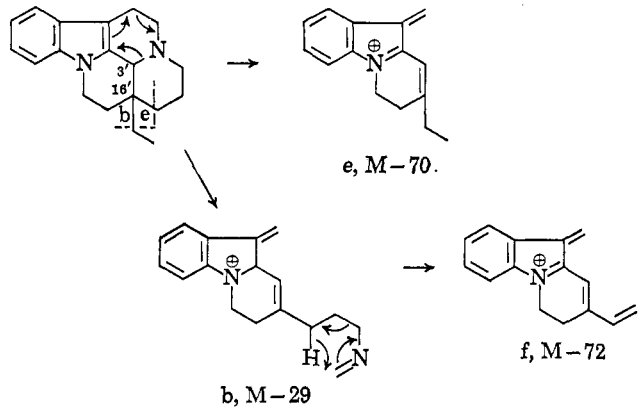
IV, R = OH (equatorial)
V, R = H

VI

constituent of *P. mutica*.[3]

Thus, on the basis of the elemental composition of the "substituent" which corresponds to eburnamenine plus one hydrogen, part structure VII was suggested as a working hypothesis for the "dimer." Carbon-14′ was anticipated as the site of substitution for biogenetic reasons, since carbomethoxyl, hydroxyl, and ketone functions in the eburnamine-type alkaloids always occur at this position. The mass spectrum of VII was expected to exhibit a fragmentation pattern similar to that produced by dihydroeburnamenine (V). The spectra of V and related compounds have been discussed in detail elsewhere,[8,9] but the fragmentation of V is briefly summarized here for comparison.

A retro-Diels–Alder reaction, together with cleavage at C-16′, leads to the two major peaks of the dihydroeburnamenine spectrum. Further rearrangement of fragment b produces an M − 72 ion. An intense M − 1 peak is probably due to loss of the C-3′ proton.



Substituted fragments equivalent to b, e, and f are observed in the spectrum of the "dimer" and thus provide excellent evidence for the presence of the dihydroeburnamenine skeleton. Remaining peaks of the spectrum bear no resemblance to spectra of pleiocarpinine or dihydroeburnamenine, and must arise by cleavage and rearrangement in the vicinity of the "dimer" linkage.

Thus, the mass spectral correlations provide strong evidence for the presence of pleiocarpinine and dihydroeburnamenine. Confirmation, including determination of sites of substitution, was obtained by the following degradations. Treatment of the "dimer" with 50% phosphoric acid at reflux temperature produced one major cleavage product. After purification by thin-layer chromatography, it was identified as pleiocarpinine on the basis of its mass spectrum. The second moiety could not be isolated intact under these reaction conditions, and was probably represented by the polar, nonvolatile material observed on the thin-layer plates near the origin. Similar results were obtained with stannous chloride in hydrochloric acid, although hydrochloric acid alone failed to cleave the alkaloid.

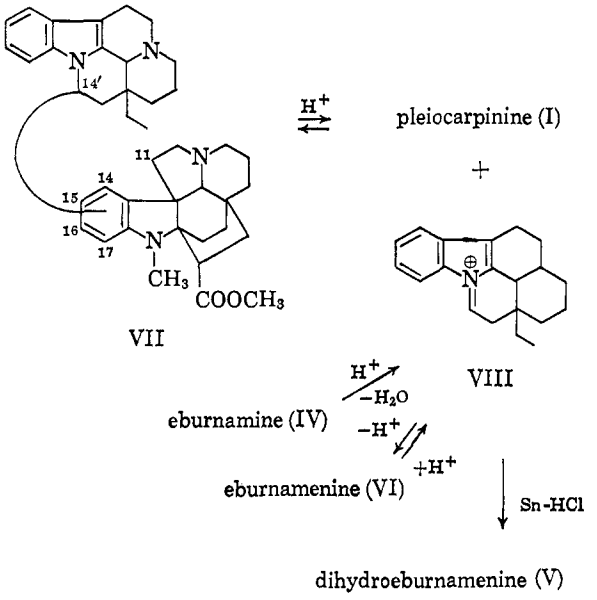
Under the conditions of acid cleavage, the dihydroeburnamenine moiety should form the ion of structure VIII, which cannot be isolated as such. However, reducing conditions could transform this ion to dihydroeburnamenine (V). As predicted, cleavage in hydrochloric acid with metallic tin yielded V together with a small amount of pleiocarpinine.

(7) (a) M. F. Bartlett and W. I. Taylor, *J. Am. Chem. Soc.*, 82, 5941 (1960); (b) E. Wenkert and B. Wickberg, *ibid.*, 87, 1580 (1965); (c) J. Trojanek, Z. Koblicova, and K. Blaha, *Chem. Ind.* (London), 1261 (1965).

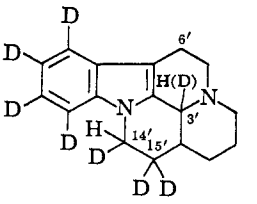
(8) M. Plat, D. D. Manh, J. LeMen, M.-M. Janot, H. Budzikiewicz, J. M. Wilson, L. J. Durham, and C. Djerassi, *Bull. Soc. Chim. France*, 1082 (1962).

(9) H. K. Schnoes, A. L. Burlingame, and K. Biemann, *Tetrahedron Letters*, 993 (1962).

In an attempt to determine the positions at which the two monomeric units are joined, the alkaloid was cleaved with deuterated acid. The isolated cleavage products should then contain a single deuterium atom at the point of substitution, in place of the second moiety. Cleavage of the alkaloid VII in phosphoric acid-d_3 indeed produced pleiocarpinine-d_2. However, this compound was mass spectrometrically identical with the product obtained by acid-catalyzed deuteration of pleiocarpinine itself, because under acidic conditions the *ortho* and *para* protons at C-15 and C-17 exchange with the solvent. Since the cleavage in phosphoric acid-d_3 led only to a dideuterio derivative, we conclude that the second alkaloidal moiety replaces one of the exchangeable hydrogens in pleiocarpinine and thus must be attached to either C-15 or C-17. If the site of substitution were C-11, 14, or 16, a trideuteriopleiocarpinine should be obtained. The exact position of substitution, C-15 or C-17, will be discussed later.



The proposed substitution at C-14′ of dihydroeburnamenine could be confirmed by cleavage with tin and hydrochloric acid-*d*. A mixture of dihydroeburnamenine-d_7 and -d_8 was obtained. The eighth deuterium was probably located at C-3′, as indicated by a strong (M − d_8) − 2 peak in the mass spectrum and absence of a (M − d_7) − 2 peak. It was expected that the four aromatic protons of the indole nucleus would exchange under acidic conditions. The partial exchange of the C-3′ proton was more unusual; however, the product could be partially back exchanged at this position when treated with hydrochloric acid. The remaining three protons were nonexchangeable and were limited to positions C-6′, C-14′, or C-15′, since all deuterium was retained in the M − 29 and M − 70 fragments of the mass spectrum. Only structure VII could readily lead to the incorporation of three nonexchangeable deuterium atoms in the cleavage product. Taking account of the cleavage conditions, structure IX may be assigned to this product. Incorporation of deuterium at C-15′ resulted from equilibration of ion VIII with solvent to yield eburnamenine (VI), and a single deuterium atom at C-14′ was introduced by reduction.



IX

The following fragmentation schemes (supported by high-resolution data, see Table I) complete the interpretation of the mass spectrum of the dimeric alkaloid in terms of structure VII.

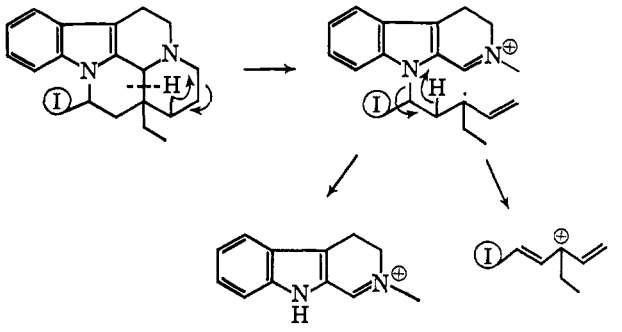
Table I. High-Resolution Mass Spectral Data for 15-(14′-Eburnamyl)pleiocarpinine (X)

	Detd	Calcd	C	H	N	O
M^+	630.3915	630.3934	41	50	4	2
a	602.3633	602.3621	39	46	4	2
b	601.3559	601.3542	39	45	4	2
c	599.3735	599.3750	40	47	4	1
d	571.3780	571.3801	39	47	4	0
e	560.3282	560.3277	37	42	3	2
f	558.3090	558.3120	37	40	3	2
g	544.3592	544.3566	37	44	4	0
h′	529.3053	529.3093	36	39	3	1
i	507.2870	507.2886	33	37	3	2
j	506.2808	506.2807	33	36	3	2
k	493.2696	493.2729	32	35	3	2
l	492.2653	492.2651	32	34	3	2
m	445.2818	445.2855	29	37	2	2
n	378.2288	378.2307	24	30	2	2
o	365.2206	365.2229	23	29	2	2
p	350.1990	350.1994	22	26	2	2
q	337.1923	337.1916	21	25	2	2
r	278.1786	278.1783	19	22	2	0
s	265.1702	265.1705	18	21	2	0
t	252.1625	252.1626	17	20	2	0
u	250.1483	250.1470	17	18	2	0
v	228.1027	228.1025	14	14	1	2
w	208.1116	208.1126	15	14	1	0
x	185.1078	185.1079	12	13	2	0
y	124.1123	124.1126	8	14	1	0
z	109.0899	109.0891	7	11	1	0

In the preceding discussion of ions related to pleiocarpinine, fragments j and k were discussed. Although not significant in the spectrum of the monomer, the analogous substituted fragments (*m/e* 506 and 493, respectively) are more pronounced in the spectrum of the "dimer." The accompanying M − 138 peak differs from k by one hydrogen which may be the C-3′ hydrogen of the dihydroeburnamenine moiety.

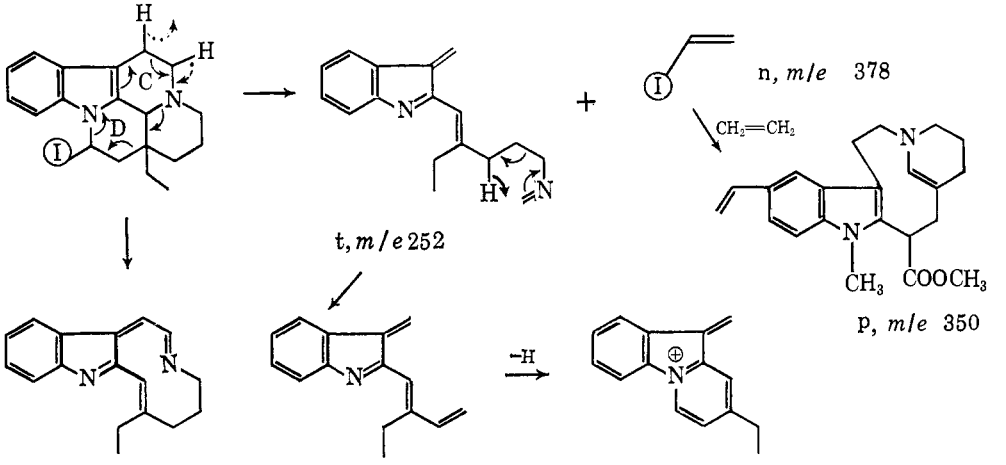
It was suggested earlier that the peak at *m/e* 529 was formed by a process similar to that leading to h in pleiocarpinine. However, this fragment is only a minor contributor to the *m/e* 529 species. The high-resolution mass spectrum shows a multiplet of low intensity, the major component (h′) of which is due to loss of OCH_3 from ion e.

The elemental composition of m (Table I) and its shift to *m/e* 446 in the spectrum of pentadeuterioeburnamylpleiocarpinine (XI) indicate this ion of mass 445 lacks the aromatic portion and both nitrogen atoms of dihydroeburnamenine. The following fragmentation processes are suggested to lead to m and x.



x, *m/e* 185 m, *m/e* 445

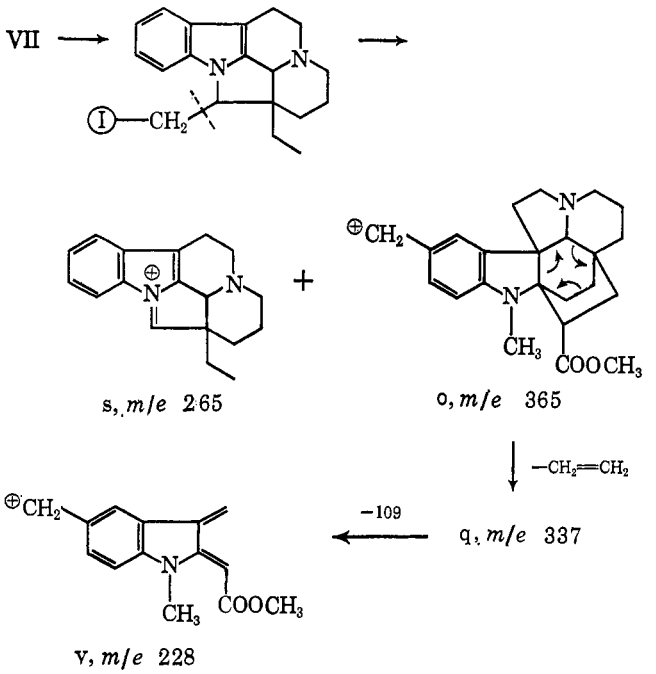
Two of the major ions of the spectrum, n and t, may be derived from a combination of two retro-Diels–Alder cleavages, opening rings C and D. A variation of this rearrangement involving loss of a hydrogen molecule (dotted arrows) leads to fragment u, *m/e* 250. Fragments p and w are derived from these major ions by the following reactions.



u, *m/e* 250 w, *m/e* 208

The peak at *m/e* 278 may correspond to the molecular ion of eburnamenine (VI), obtained by a 1,2-elimination reaction. Peaks at *m/e* 249 and 208 are present, but are of weaker relative intensity than in the mass spectrum of eburnamenine itself; yet this is not inconsistent, as the eburnamenine molecular ion obtained by a rearrangement process may not contain sufficient energy to fragment further.

Transfer of a C-15′ proton with contraction of ring D leads to an isomeric form of the molecular ion, which produces stable fragments s and o by simple cleavage. Two major fragmentation processes of pleiocarpinine then lead to the ion of mass 228.



s, *m/e* 265 o, *m/e* 365

q, *m/e* 337

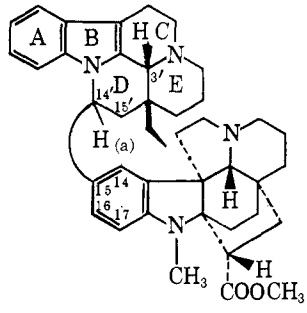
v, *m/e* 228

While the foregoing discussion of the spectral and chemical evidence for structure VII is rather convincing, the synthesis of that one of the four isomers of VII which is identical with the natural product was desirable to definitely settle the question of attachment and stereochemistry, as well as provide unambiguous proof of its structure. Precedent for the successful preparation of "dimeric" indole alkaloids was available in the partial syntheses of voacamine, voacamidine, and voacorine.[10] These compounds have the same type of carbon–carbon linkage as the proposed structure for our alkaloid: the carbon atom α to an indole system attached directly to the aromatic ring of a second indole alkaloid. Under acidic conditions, a carbinol–indole precursor formed an iminium ion which condensed readily with the nucleophilic aromatic ring of a second alkaloid present in the reaction mixture. Thus, in the analogous case of the "dimer" VII, it was expected that eburnamine (IV) might form the intermediate iminium ion VIII which could undergo electrophilic substitution with pleiocarpinine (I). Up to four isomers might result, differing in stereochemistry at C-14′ or in point of substitution at the aromatic ring.

To effect this synthesis, a mixture of equal amounts of pleiocarpinine (I) and eburnamine (IV) was refluxed in dilute hydrochloric acid. A single product was isolated in 74% yield by thin-layer chromatography, followed by crystallization, which was identical with the natural product (identical in R_f value, nmr, ultraviolet, infrared, and mass spectra; mixture melting point not depressed). In particular, the identical nmr absorption at 5.0 ppm and in the aromatic region demonstrated the same type of linkage (discussed in detail later) in the synthetic and natural products.

(10) G. Büchi, R. E. Manning, and S. A. Monti, *J. Am. Chem. Soc.*, **86**, 4631 (1964).

The formation of only one product can be accounted for by steric considerations. Treatment of eburnamine (IV) with acid previously led to an equilibrium mixture of eburnamine (90%) and 14′-isoeburnamine (10%).[7a] This ratio of products had suggested that the hydroxyl group of eburnamine was equatorial, and that the axial position was sterically hindered by the ethyl group. Thus it seemed that the greater size of the pleiocarpinine moiety of VII, relative to the hydroxyl group of eburnamine, would dictate a single isomer at C-14′ for the dimeric alkaloid, with the same relative stereochemistry as eburnamine. In addition, substitution at C-15 of the pleiocarpinine moiety should be preferred (*e.g.*, structure X), since the N-methyl group would seriously interfere with the eburnamyl substituent at the alternative position C-17.

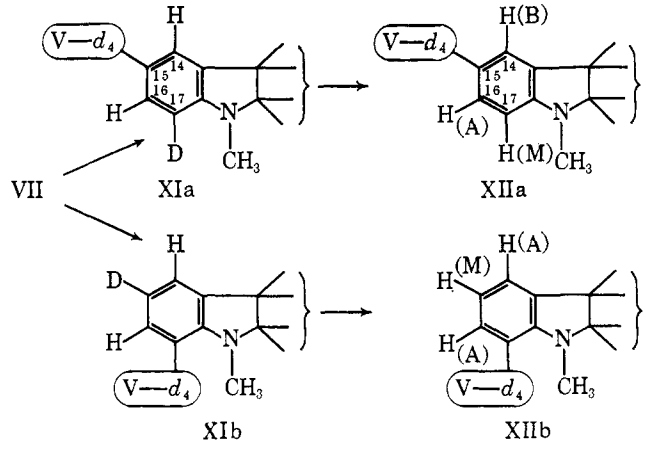


X

The suggested structure (X) was confirmed by nmr studies. The proton at C-14′ in eburnamine (IV) gives rise to a quartet at 5.4 ppm (double–doublet, J = 5 cps and J = 10 cps) resulting from coupling with each of the C-15′ protons. The coupling constants between axial–axial (a–a) protons is usually much larger than the constants of axial–equatorial (a–e) or equatorial–equatorial (e–e) couplings, the latter two being of approximately the same magnitude. The observed splittings in the eburnamine spectrum are consistent only with a–e and a–a couplings, respectively, and the single hydrogen at C-14′ must therefore be axial. This is in agreement with the results of the eburnamine–isoeburnamine equilibrium[7a] mentioned above. The nmr spectrum of the "dimeric" alkaloid shows a one-proton quartet at 5.0 ppm (J = 5 cps and J = 11 cps), and it must, therefore, have the same relative stereochemistry as eburnamine. The relative stereochemistry of the D–E ring junction,[7b] as well as the absolute stereochemistry at C-3′ of the eburnamine-type alkaloids,[7c] has recently been determined. However, the stereochemistry at C-14′, relative to the ethyl group, in eburnamine (and thus in eburnamylpleiocarpinine (X)) remains uncertain.

The second linkage position was identified after simplification of the nmr spectrum by selective exchange of some of the aromatic hydrogens with deuterium. Treatment of the "dimer" with 4 *N* deuterium chloride produced a pentadeuterio derivative, either XIa or XIb. Complete exchange of the indole hydrogens was evident from a shift by four mass units of mass spectral peaks r, s, t, u, w, and x. The fifth deuterium could be assigned to the aromatic nucleus of I, deduced by the shift of peaks m, n, o, p, q, and v by one mass unit. In this product the aromatic region of the nmr spectrum (obtained in acetone) was simplified to a two-proton signal, which appeared as a sharp singlet at 7.16 ppm. Thus the C-14 and C-16 hydrogens of XI are equivalent, and their coupling cannot be detected.

Having established this fact, it was of interest to reintroduce the third aromatic proton of the pleiocarpinine moiety and to observe the multiplicity of its nmr signal as well as changes in the signal of the *meta* protons at C-14 and C-16. Reflux of XI in 1% hydrochloric acid for 15 min resulted in the loss of only one deuterium atom. The product was identified as either XIIa or XIIb, since peaks r, s, t, u, w, and x retained the four mass unit shift observed for XI, whereas m, n, o, p, q, and v now were found at the same positions as in the mass spectrum of the unlabeled dimer.



The following predictions of the nmr spectra of XIIa and XIIb are based on simple splitting rules, with full understanding that a more complex pattern could be observed, depending on relative chemical shifts and coupling constants. However, it seemed likely that the third hydrogen would be sufficiently different from the *meta* hydrogens so that first-order rules might apply.

Structure XIIb resembles an A_2M system, since the addition of the C-15 proton should not affect the equivalence of the *meta* hydrogens which was observed in XI. In absence of complications, the spectrum would show a 2 H doublet and a 1 H triplet, with *ortho* coupling constants of 6–10 cps.

An ABM system is represented by XIIa, in which the *para* coupling J_{BM} should be too small to be observed. Thus $H_{(M)}$ and $H_{(B)}$ would appear as doublets, and $H_{(A)}$ as a quartet. The actual spectrum of XII (Figure 3) may be in fact interpreted perfectly in terms of structure XIIa by this prediction. Chemical shifts are: A = 7.15 ppm, B = 7.14 ppm, M = 6.53 ppm, with coupling constants J_{AM} = 8.5 cps and J_{AB} = 2.0 cps. These data complete the assignment of structure X for the new alkaloid.

Since both monomeric units of X are also constituents of *P. mutica* Benth, the question might arise whether the "dimer" could be an artifact, formed during extraction and isolation. In the isolation procedure we had employed only chromatography of the extract without prior separation of the basic fraction, and thus avoided the use of acidic conditions required for such a condensation. In our opinion, the compound is therefore not likely to be an artifact.

Experimental Section

Reaction products were isolated and purified by small-scale preparative chromatography on thin-layer silica gel H, prewashed with methanol. Chloroform–methanol (9:1) was the developing system, and spots were detected with iodine vapor.

Ultraviolet spectra were determined in methanol using a Cary Model 14 recording spectrophotometer, and infrared spectra were obtained in chloroform with a Perkin-Elmer Model 337 spectrophotometer. Conventional mass spectra were determined with a CEC 21-103C mass spectrometer, equipped with a direct inlet system. High-resolution data were obtained with a CEC 21-110 double focusing mass spectrometer, using a photographic plate for recording.

Complete nmr spectra in deuteriochloroform, with tetramethylsilane (TMS) as internal standard, were determined with a Varian A-60 spectrometer. Partial nmr spectra of the aromatic region were obtained in acetone, using TMS as a standard. A Varian C-1024 time-averaging computer was used to increase the signal to noise ratio of weak spectra.

Isolation of Eburnamylpleiocarpinine (X). A methanol extract of the bark of *P. mutica* Benth, which had been depleted of pleiocarpine by crystallization, was chromatographed (in two portions of 130 g each) on alumina and silicic acid.[2b] Fractions C233 to C235 were crystallized from methanol to give about 1 g of X. The following data were obtained after two recrystallizations from MeOH–$CHCl_3$ (9:1): mp 225° dec; ν_{max} $CHCl_3$ 1725, 1610 cm^{-1}; λ_{max} MeOH 230 mμ (log ε 4.50), 263 (4.20), 285 (3.97), 292 (3.97); nmr 0.9 (3 H triplet), 2.8 (3 H singlet, N-CH_3), 3.8 (3 H singlet, O-CH_3), 5.0 ppm (1 H quartet), multiplet (7 H) in the region of 6.2–7.5 ppm; mass spectrum Figure 1 and Table I; $[\alpha]^{26}D$ −111° (*c* 1.930, $CHCl_3$).

Lithium Aluminum Hydride Reduction of X. The "dimer" X (*ca.* 10 mg) was refluxed for 3 hr in tetrahydrofuran with excess lithium aluminum hydride. After evaporation to dryness, potassium sodium tartrate solution was added and the product was extracted with chloroform. The ultraviolet spectrum was identical with that of X. The infrared spectrum showed hydroxyl absorption at 3350 cm^{-1}, and absence of a carbonyl group. Mass spectrum: *m/e* 602 (M^+), 584, 574 (a), 573 (b), 571, 557, 545, 544 (g), 532 (e), 530 (f), 518, 478 (j), 464 (l), 417 (m), 350 (n), 337 (o), 323, 309 (q), 301 (M^{2+}), 287 (a^{2+}), 278 (r), 265 (s), 252 (t), 250 (u), 208 (w), 200 (v), 185 (x), 124 (y), 109 (z).

Lithium Aluminum Deuteride Reduction of X. The same procedure was followed for the lithium aluminum deuteride reduction of X: mass spectrum *m/e* 604 (M^+), 586, 576 (a), 575 (b), 571, 559, 547, 544 (g), 534 (e), 532 (f), 520, 480 (j), 466 (l), 419 (m), 352 (n), 339 (o), 325, 311 (q), 302 (M^{2+}), 288 (a^{2+}), 278 (r), 265 (s), 252 (t), 250 (u), 208 (w), 202 (v), 185 (x), 124 (y), 109 (z).

Phosphoric Acid Cleavage of X to Pleiocarpinine (I). Compound X (10 mg) was refluxed for 30 min in 1 ml of 45% phosphoric acid. The solution was neutralized with sodium carbonate solution and extracted with chloroform. Pleiocarpinine (I) was the major cleavage product, isolated by thin-layer chromatography and identified by its mass spectrum. The small yield of I precluded crystallization.

Stannous Chloride Cleavage of X to Pleiocarpinine (I). Reflux of 20 mg of X in 2 ml of 20% hydrochloric acid with 20 mg of stannous chloride for 30 min, followed by the isolation procedure described above, yielded pleiocarpinine (I) as the major detectable cleavage product.

Deuteriophosphoric Acid Cleavage of X to Pleiocarpinine-d_2. Treatment of the dimeric alkaloid X for 30 min with 50% phosphoric acid-d_3, prepared by addition of phosphorus pentoxide to deuterium oxide, yielded pleiocarpinine-d_2. All major peaks of the mass spectrum, except *m/e* 124 and 109, were shifted by two mass units. Treatment of pleiocarpinine (I) with phosphoric acid-d_3 under the same cleavage conditions also led to pleiocarpinine-d_2 with an identical mass spectrum.

Reductive Cleavage of X to Dihydroeburnamenine (V). The "dimer," 30 mg, was refluxed for 4 hr in 5 ml of 4 *N* hydrochloric acid with 200 mg of tin powder. After neutralization with sodium carbonate and extraction with chloroform, thin-layer chromatography indicated one major product, with only traces of pleiocarpinine and starting material. A mass spectrum of this product was identical with the spectrum of dihydroeburnamenine (V), with small peaks at *m/e* 109 and 124 due to a trace of pleiocarpinine.

Reductive Cleavage of X to Dihydroeburnamenine-d_7 and -d_8 (IX). Tin powder (200 mg) and 20 mg of X were refluxed for 4 hr in 2 ml of *ca.* 4 *N* D^+, prepared from 280 mg of phosphorus pentachloride and 2 ml of deuterium oxide. After work-up as described above, deuterated dihydroeburnamenine (IX) was isolated. The mass spectrum had peaks at *m/e* 288 (M-d_8), 287 (M-d_7), 286 ((M-d_8) − D plus (M-d_7) − H), 259 and 258 (b), 218 and 217 (e). Calculations based on the deuterium content of fragments b and e (because of the strong M − 1 peak) indicated the approximate ratios $d_8/d_7/d_6$ = 100:70:10.

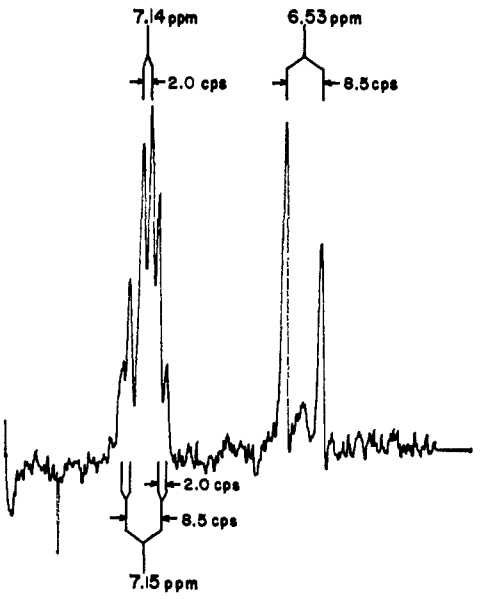


Figure 3. Partial nmr spectrum of 15-(14′-eburnamyl)pleiocarpinine-d_4 (XIIa).

Conversion of IX to Dihydroeburnamenine-d_3. The material obtained in the previous experiment was refluxed for 3 hr in 1 ml of aqueous 4 *N* hydrochloric acid, then neutralized and extracted. The mass spectrum indicated the major species to be dihydroeburnamenine-d_3, *m/e* 283, with a small amount of the tetradeuterio derivative present. The (M-d_3) − 2 peak was relatively small, implying that none of the three deuterium atoms was located at C-3′. Other major mass spectral peaks were at *m/e* 254 (b) and 213 (e).

Deuterium Exchange of X to Eburnamylpleiocarpinine-d_5 (XI). The natural dimeric alkaloid X (100 mg) was refluxed for 2 hr in 10 ml of *ca.* 4 *N* D^+ prepared from phosphorus pentachloride and deuterium oxide. Neutralization, extraction, and chromatography yielded 13 mg of XI. More polar material was present as well as some cleavage products. Partial mass spectrum of XI: *m/e* 635 (M^+), 446 (m), 379 (n), 256 (t), with other peak shifts consistent with structure XI. The nmr spectrum in acetone, after ten scans with the time-averaging computer, showed a singlet at 7.16 ppm, with no other absorption present in the aromatic region.

Hydrogen Exchange of XI to Eburnamylpleiocarpinine-d_4 (XII). The total sample of XI was refluxed for 15 min in 1 ml of 1% hydrochloric acid. Neutralization and extraction yielded 10 mg of the tetradeuterio derivative XII. Mass spectrum: *m/e* 634 (M^+), 445 (m), 378 (n), 256 (t). The nmr spectrum in acetone is given in Figure 3, determined with 50 computer scans, 100-cps sweep width, and 50 sec/scan.

Partial Synthesis of Eburnamylpleiocarpinine (X). A mixture of 40 mg of pleiocarpinine (I) and 40 mg of eburnamine (IV) was refluxed for 8 hr in 8 ml of 2% aqueous hydrochloric acid. The solution was neutralized with sodium carbonate and extracted with chloroform. Preparative thin-layer chromatography gave 53 mg of eburnamylpleiocarpinine (X). Crystallization from chloroform–methanol yielded 22 mg of X, mp 218° dec. A mixture of synthetic and natural products melted with decomposition at 218°. The synthetic alkaloid gave nmr, infrared, ultraviolet, and mass spectra which were identical with spectra of the natural product. A single (more polar) minor product contained no ester group (infrared) and was therefore not an isomer of X. Rotation for synthetic X was $[\alpha]^{27}D$ −102° (*c* 1.477, $CHCl_3$).

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the identity of eburnamylpleiocarpinine and pleiomutine. This investigation was supported by a Public Health Service fellowship (5-F1-GM-21, 624-03) from the National Institute of General Medicine Sciences (to D. W. T.) and a research grant from the National Science Foundation (GP-3734).

Elucidation of the Structures of the Sapogenins of *Polygala senega* by Correlation with Medicagenic Acid[1,2]

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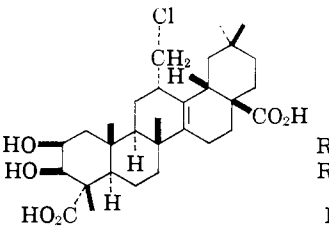
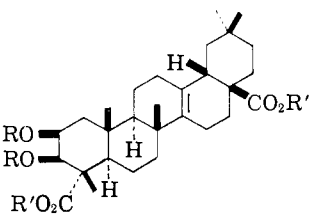
Abstract: A postulated precursor of senegenic acid (**2**), "hydroxysenegenin" (**6**), was isolated by the dilute sulfuric acid hydrolysis of senegin, but proved not to be a precursor. Another presumed precursor of senegenin (**1**), cyclosenegenin (**9**), was obtained by the action of alkali on senegenin. Hydrolysis of cyclosenegenin with hydrochloric acid affords senegenin as predicted, but its presence in the saponin, senegin, is not indicated. The structures of the real precursor, presenegenin (**17**), and its artifacts were confirmed by a direct correlation with medicagenic acid by a five-step sequence. This correlation establishes certain stereochemical features which were based on biogenetic analogy to other terpenes.

Extracts of *Polygala senega* L. (Polygalaceae) have been used as an expectorant for centuries. The main constituent is the saponin "senegin," which on hydrolysis with hydrochloric acid affords two crystalline sapogenins: senegenin and senegenic acid.[4a] Recent work has resulted in the assignment of structures[4b] **1** and **2** to senegenin and senegenic acid, respectively.[5-7] Since senegenin contains chlorine and senegenic acid has one less carbon than **1**, it was suspected that both compounds are artifacts produced during the hydrochloric acid treatment. In the course of a search for the precursor of these artifacts two new senega compounds were isolated. This paper describes the isolation and structure determination of these compounds as well as the correlation of the genuine precursor, presenegenin, with medicagenic acid.

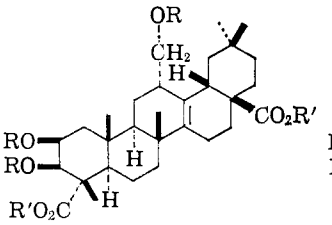
In an effort to explore the effect of milder hydrolytic conditions on senegin, the saponin was treated briefly with 2 *N* aqueous sulfuric acid. The water-insoluble product was acetylated and chromatographed on a silica gel column. Two crystalline acetates were isolated,[8] one of which was identified as senegenic acid diacetate (**3**).[6,7] The other acetate is assigned structure **5** on the following evidence. It has the formula $C_{36}H_{52}O_{10}$, and is hydrolyzed to an acetyl-free compound, $C_{30}H_{46}O_7$ (**6**) which regenerates (**5**) upon reacetylation. The three hydroxyl groups of **6** are acetylable, since **5** shows no hydroxyl absorption in the infrared. The pmr spectrum of **5** exhibits three acetyl groups at τ 8.04, 7.94, 7.92 besides five C-methyl signals. Signals at τ 4.66 (1 H, doublet, J = 4 cps) and 4.44 (1 H, broad) are very similar to those exhibited by the diacetates of senegenin and senegenic acid, suggesting the presence of 2β,3β-diacetoxy groups[5a,6] in compound **5**. The ill-defined AB system at τ 6.10 and 5.65 (J = 11 cps) is probably due to an acetoxy methylene group attached to an asymmetric center.[9] Methylation of **5** with diazomethane gave a noncrystalline dimethyl ester (**7**) which shows two



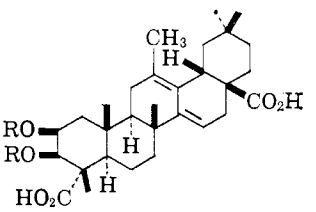
1



2, R = R′ = H
3, R = Ac; R′ = H
4, R = H; R′ = CH_3



5, R = Ac; R′ = H
6, R = R′ = H
7, R = Ac; R′ = CH_3



8, R = H
14, R = Ac

(1) This investigation was supported in part by Grant GM 10966 from the National Institutes of Health, U. S. Public Health Service.

(2) Preliminary accounts of this work were outlined in communications: (a) Y. Shimizu and S. W. Pelletier, *J. Am. Chem. Soc.*, 87, 2065 (1965); (b) *Chem. Ind.* (London), 2098 (1965).

(3) To whom inquiries regarding this paper should be addressed.

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(7) S. W. Pelletier, N. Adityachaudhury, M. Tomasz, J. J. Reynolds, and R. Mechoulam, *J. Org. Chem.*, 30, 4234 (1965).

(8) A very small amount of nonterpenoid crystals was also obtained. This proved to be a mixture of 4-methoxycinnamic acid and 3,4-dimethoxycinnamic acid (see the Experimental Section).

(9) L. M. Jackmann, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press Inc., New York, N. Y., 1959, p 102.