The Structure of Alstonisidine, a Novel Dimeric Indole Alkaloid from *Alstonia muelleriana* Domin

JAMES M. COOK AND P. W. LE QUESNE*[1]

Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48104

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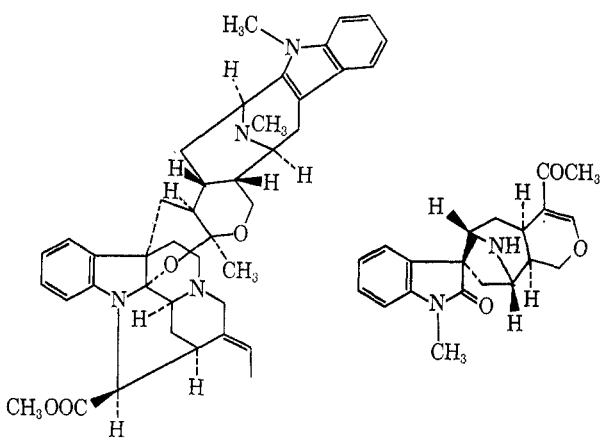
Alstonisidine, a "dimeric" indole alkaloid from the aerial bark of *Alstonia muelleriana* Domin, is assigned structure **5** on the basis of chemical reactions and spectral data. The two "monomeric" units of alstonisidine resemble macroline, **7**, and quebrachidine, **13** (stereochemistry is not assigned), and are linked in a novel manner. Possible biogenetic relationships with related alkaloids are discussed.

In their investigation of the alkaloids of the aerial bark of the Australian tree *Alstonia muelleriana* Domin, Elderfield and Gilman[2] isolated four compounds in pure form: villalstonine (**1**)[3,4] (= alkaloid B),[2a,b] alstonisine (**2**)[2c,5] (= alkaloid C),[2a,b] alstonerine (**3**),[6] and alstonisidine[2c] (= alkaloid A).[2a,b] Macralstonine (**4**)[7] has also recently been obtained.[8] We now report our work on a small sample of alstonisidine kindly made available by Professor Elderfield and propose structure **5**. This structure, while of a type not previously encountered, is in good accord with all evidence available, and is consonant with the likely biogenesis of related alkaloids.[9]



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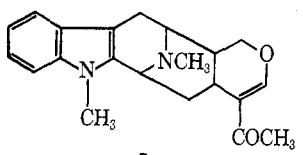

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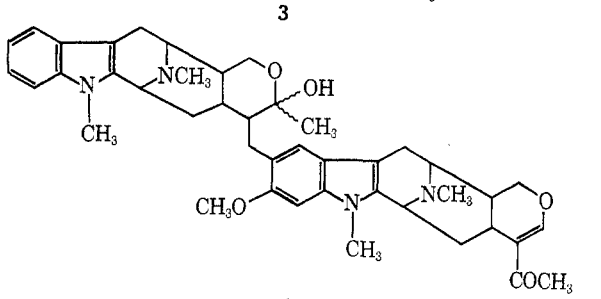

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Alstonisidine, $C_{42}H_{48}N_4O_4$, $[\alpha]D$ −234° (ethanol), shows λ_{max}^{EtOH} 230, 286, 294 nm (ϵ 41,600, 12,000, 12,100), λ_{min} 252 sh, 265 (ϵ 12,100), which is very similar to the uv spectrum of villalstonine (**1**),[4] and indicates the presence of indole and indoline chromophores. The ir spectrum lacks an N–H peak, but in dilute solution in carbon tetrachloride shows a weak, broad absorption at 3090 cm^{-1}, arising from a hydrogen-bonded alcoholic –OH group. Peaks at 1735, 1610, 1470, 1450, and 1380 cm^{-1} are assigned to carboxylic ester, indoline, indole, and C–CH_3 functions. The nmr spectrum shows a 7H multiplet centered at τ 2.95, arising from the indole and indoline groups, and indicates the absence of α or β protons on the indole nucleus.[10] The vinyl proton of an ethylidene group falls at τ 4.78 (q, J = 7 Hz), and the corresponding methyl signal at τ 8.28 (d, J = 7 Hz). A 3H singlet at τ 6.28 is assigned to an indolic NCH_3 group, and a 3H singlet at τ 6.37 to a carbomethoxy group. An aliphatic NCH_3 signal falls at τ 7.63. The highest field signal in the spectrum is a 3H singlet at τ 8.50, which is assigned to the amino-ketal quaternary methyl group of structure **5**. These assignments are similar to those for villalstonine (**1**).[4]

The high-resolution mass spectrum of alstonisidine substantiated the molecular formula obtained by microanalysis[2c] (M^+ at m/e 672.366; calcd for $C_{42}H_{48}N_4O_4$, 672.368) and, when considered in detail in combination with other data, leads to structure **5**. The base peak in the spectrum, at m/e 197.105 (calcd for $C_{13}H_{13}N_2$, 197.108), is due to the ion **6**, which is characteristic of alkaloids containing a unit derivable from macroline (**7**).[4,6,11,12] Two other characteristic fragments are also prominent: one group of peaks at m/e 182, 181, and 180 [compare, for example, villalstonine (**1**)[4]], and a peak at m/e 170.094 (calcd for $C_{12}H_{12}N$, ion **8**, 170.097). Two small peaks, at m/e 308.185 (calcd for $C_{20}H_{24}N_2O$, 308.189) and m/e 307.183 (calcd for $C_{20}H_{23}N_2O$, 307.181) correspond with two at the same values from villalstonine (**1**)[4] and macralstonidine (**9**)[12]

(1) To whom inquiries should be addressed.

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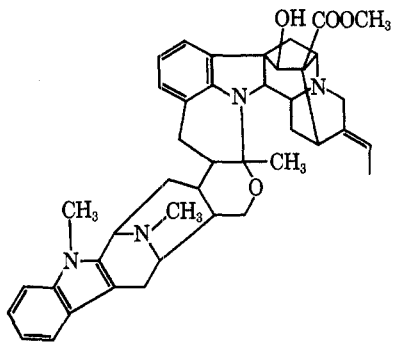

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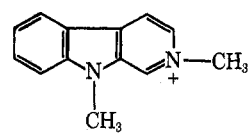

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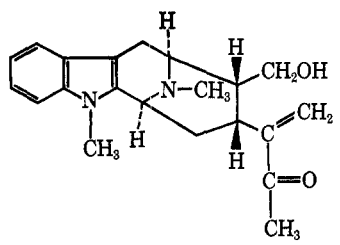

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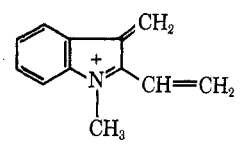

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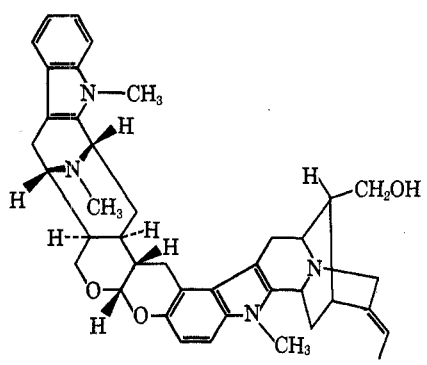
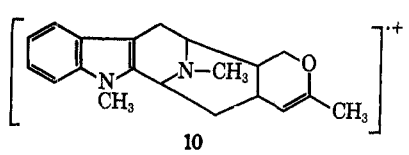


9

and are due to the fragments **10** and **11**. Fragment **10** arises by retro-Diels–Alder cleavage of alstonisidine



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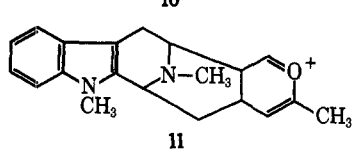


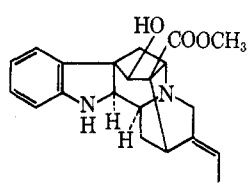
11

(**5**) (see Scheme I) and fragment **11** by loss of a hydrogen from **10** to its complementary fragment, giving the ion **12** at *m/e* 365.185 (calcd for $C_{22}H_{25}N_2O_3$, 365.187).

These data clearly prove the incorporation of an unsubstituted, ring-closed macroline unit into alstonisidine. The carbomethoxy, alcohol, and ethylidene functions are therefore associated with the indoline chromophore in the nonmacroline portion. The single quaternary C–CH_3 group of alstonisidine must, from its relatively low chemical shift, be associated with an amino-ketal or ketal function, as in villalstonine (**1**)[4] and macralstonidine (**9**).[12] At this point also, we may infer that, as in these two alkaloids, the amino-ketal or ketal function involving the quaternary C–CH_3 group forms part of the linkage between the macroline and nonmacroline portions of alstonisidine (see below).

Alstonisidine gives a mono-*O*-acetate (M^+ 714; calcd for $C_{44}H_{50}N_4O_5$, 714; ν_{max} 1740, 1735 cm^{-1}, no O–H remaining in the ir spectrum) with acetic anhydride–pyridine at 37°; this shows that, of the four oxygen atoms of the alkaloid, three are associated with the carbomethoxy and alcohol functions. That remaining is therefore the ethereal oxygen atom associated with the macroline portion and seen in the two fragment ions **10** and **11** discussed above. The quaternary C–CH_3 group must be associated with this oxygen, and hence also with a nitrogen of the nonmacroline portion, in an amino-ketal group. This is confirmed by the reduction of alstonisidine with excess lithium aluminum hydride to a triol (M^+ of triacetate at *m/e* 772; calcd for $C_{47}H_{56}N_4O_6$, 772), under conditions in which macralstonidine (**9**) is unaffected but villalstonine (**1**) gives the known villalstoninetriol.[4]

Insight into the structure of the nonmacroline portion of alstonisidine is given by the observation of a group of peaks at *m/e* 222, 221, and 220 in the mass spectrum. These are characteristic of indoline alkaloids in the ajmaline series, such as quebrachidine (**13**),[13] vincamajine (**14**),[14] and *O*-benzoylvincamajine (**15**)[15] (at *m/e* 326). These peaks represent the aliphatic portion of the indoline moiety (*m/e* 222.106, calcd for $C_{12}H_{16}NO_3$, 222.113; *m/e* 221.105, calcd for $C_{22}H_{15}NO_3$, 221.105; *m/e* 220.098, calcd for $C_{12}H_{14}NO_3$, 220.097). The $C_{12}H_{16}NO_3$ fragment has the structure **16**. The complementary fragment to **16**, which in quebrachidine



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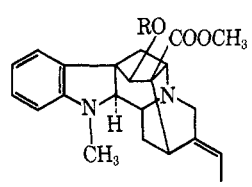
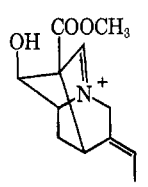


14, R = H
15, R = OCOPh



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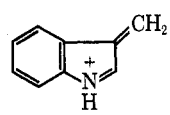

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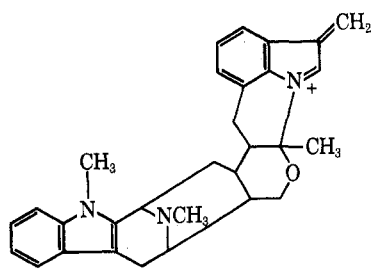

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(13) M. Gorman, A. L. Burlingame, and K. Biemann, *Tetrahedron Lett.*, No. 1, 39 (1963).

(14) M.-M. Janot, J. Le Men, J. Gosset, and J. Levy, *Bull. Soc. Chim. Fr.*, 1079 (1962); J. Trojanek and J. Hodkova, *Collect. Czech. Chem. Commun.*, **27**, 2981 (1962).

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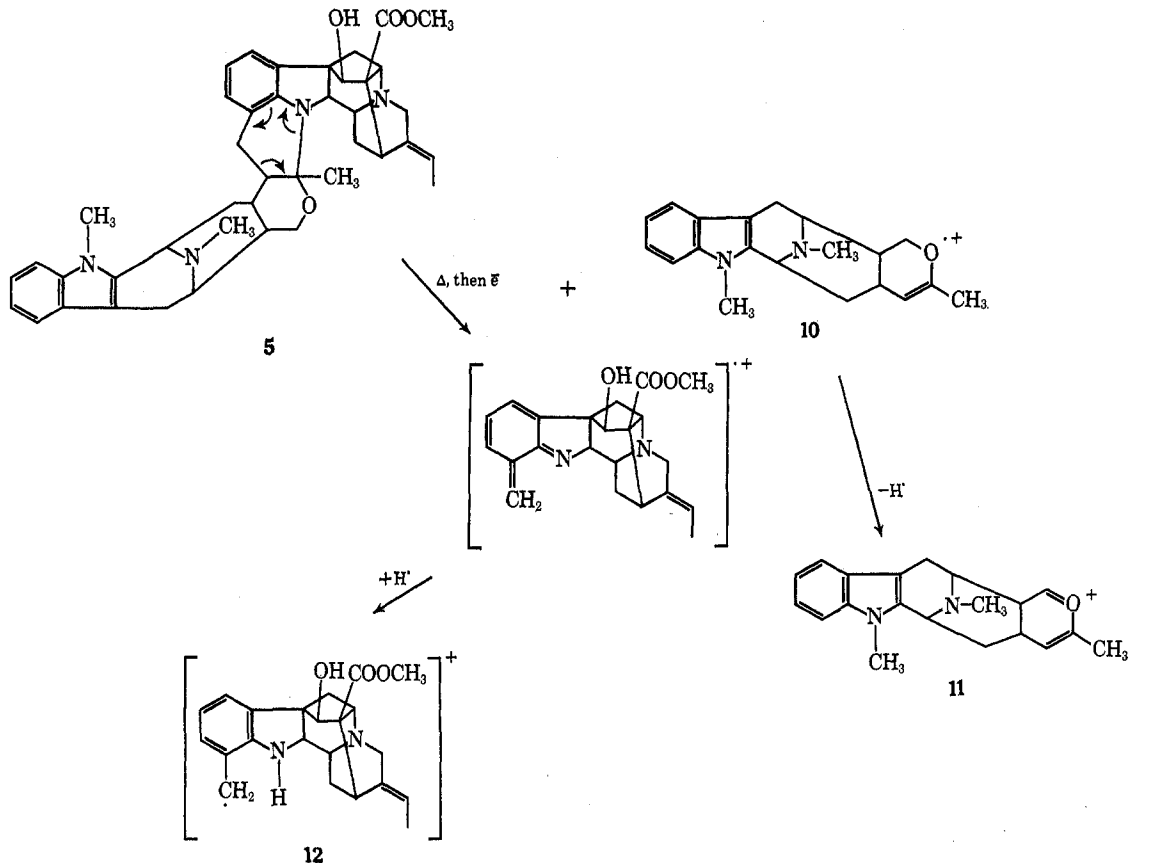
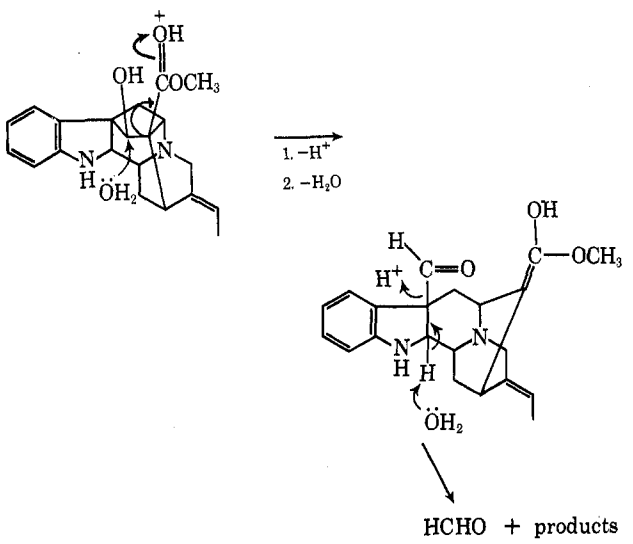
SCHEME I



SCHEME II



is the well-known fragment **17**,[13,15,16] in the spectrum of alstonisidine falls at m/e 450.252 (calcd for $C_{30}H_{32}N_3O$, 450.254) and is assigned structure **18**.

Both alstonisidine and quebrachidine under reflux with 6 *N* HCl give formaldehyde, detected by the chromotropic acid test.[12,17] A possible mechanism for this reaction is shown in Scheme II for quebrachidine.[18] Further strong similarities between alstonisidine and quebrachidine are apparent from their mass spectra.

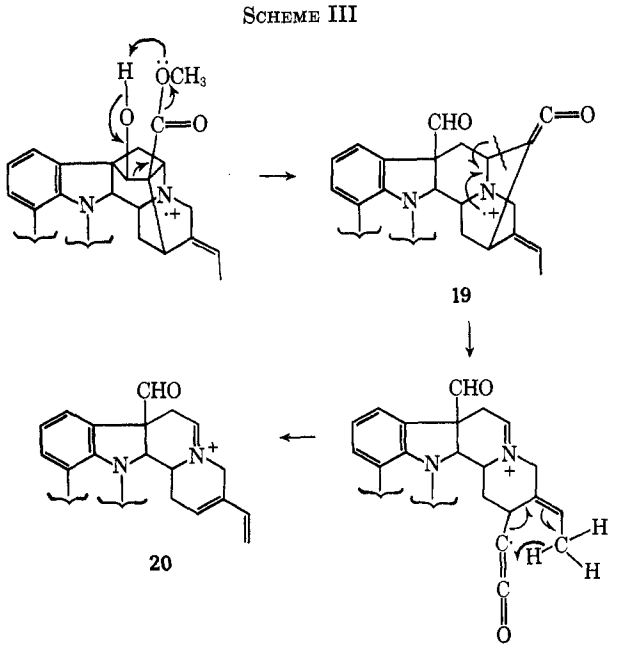
(16) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, "Alkaloids," Holden-Day, San Francisco, Calif., 1964, p 43.

(17) F. Feigl, "Spot Tests," Elsevier, Amsterdam, 1956, p 331.

(18) Further investigations of this reaction are in progress.

Although alstonisidine is readily acylated (see above), there is virtually no loss of water or OH on electron impact. This is also true for quebrachidine. As well, both mass spectra show significant peaks at M − 31 and M − 32, of which the latter is the more intense. In alstonisidine, the M − 32 peak has m/e 640.342 (calcd for $C_{41}H_{44}N_4O_3$, 640.341), establishing that CH_3OH has been lost. This is in accord with adjacency of the $COOCH_3$ and OH groups. A possible mechanism is shown in Scheme III. Loss of methanol

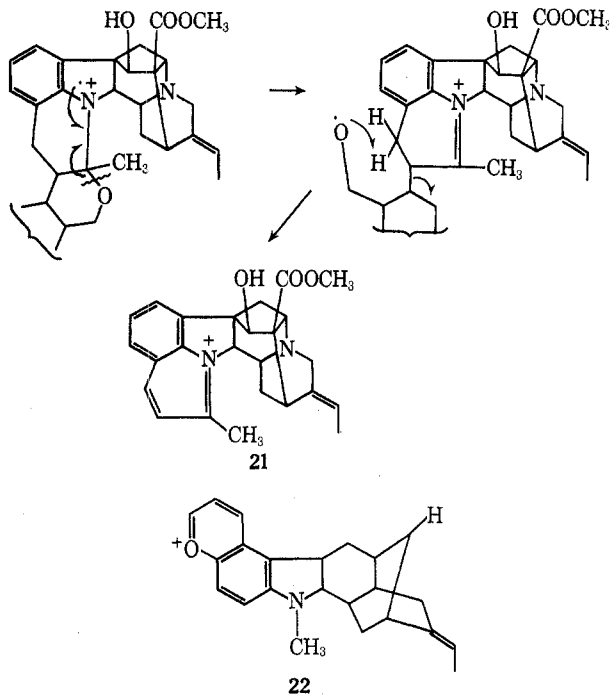
SCHEME III



from alstonisidine gives the fragment **19**, which undergoes bond cleavage as indicated. Loss of C_2HO as shown[19] gives the fragment **20**, which is seen as a small peak at *m/e* 599.342 (calcd for $C_{39}H_{43}N_4O_2$, 599.339).

Structure **5** for alstonisidine is further strongly supported by the presence of a prominent peak in the mass spectrum at *m/e* 403.202 (calcd for $C_{25}H_{27}N_2O_3$, 403.202). This peak is assigned structure **21** and its proposed origin is shown in Scheme IV. Cleavage

SCHEME IV



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and bond rotation as shown, followed by further aromatization, give the relatively stable fragment **21**, which is strikingly analogous in structure and mode of formation to the ion **22** from macralstonidine **9**.[12]

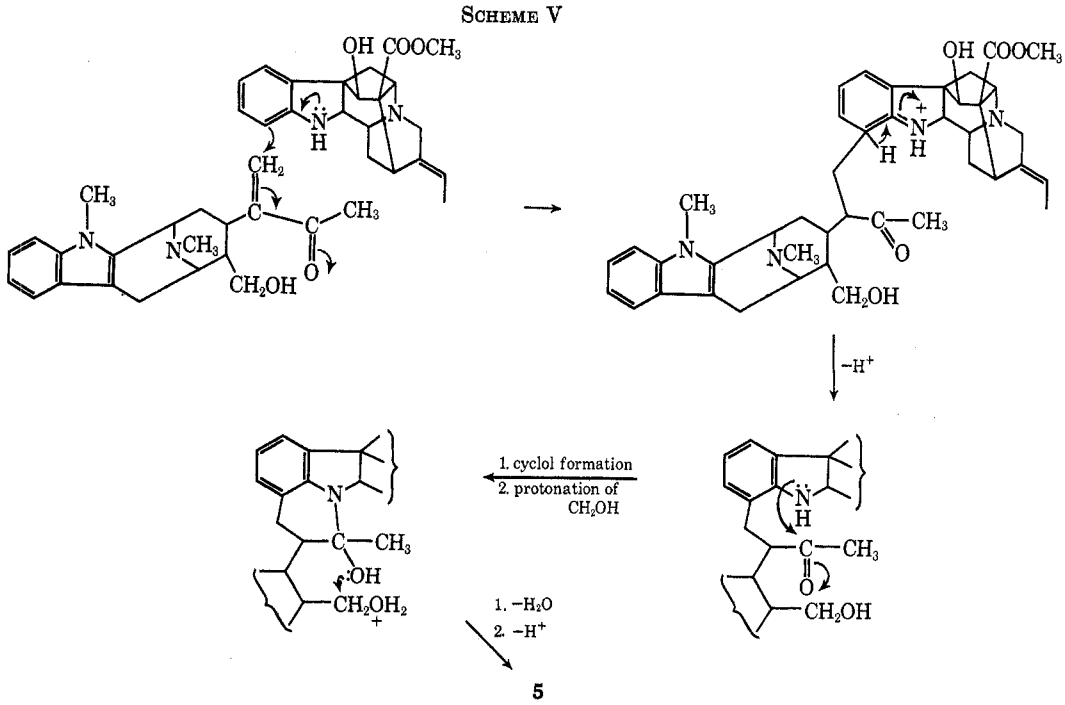
The structure **5** for alstonisidine is of considerable biogenetic interest. The work of Schmid and his coworkers[4,7,11,12] and the recent establishment of structure **3** for alstonerine[6] imply a central role for macroline **7** in the biogenesis of villalstonine (**1**),[4] macralstonine (**4**),[7] and macralstonidine (**9**).[12] Alstonisidine can be envisaged as arising (Scheme V) by electrophilic attack on the indoline aromatic ring of a quebrachidine-like species by the $CH_2{=}CC(=O)$ function of macroline (**7**). Subsequent ring closures would generate the amino-ketal function. It is noteworthy that *O*-benzoylvincamajine (**15**) has recently been obtained from the leaves of the closely related *A. macrophylla*.[15]

The stereochemistry of alstonisidine is being investigated crystallographically by Professor C. E. Nordman. Further chemical work in this area is in progress.

Experimental Section

Alstonisidine.—Alstonisidine (30 mg), obtained from Professor R. C. Elderfield, was recrystallized from methanol to give rods, mp 325° dec (uncorr). It was homogeneous on tlc,[8] and the ir spectrum was superimposable on that described by Gilman.[2a] Optical rotation and uv spectrum are also given by Gilman.[2a] The nmr data ($CDCl_3$) are given fully above. Mass spectrum was obtained by direct inlet at 325° on A.E.I. MS-902: *m/e* 674 (6.4), 673 (23), 672 (50), 658 (14), 657 (30), 641 (7), 640 (8.5), 625 (3.9), 614 (2.5), 514 (2), 484 (3.2), 470 (1), 463 (5.8), 462 (4.5), 450 (2.6), 429 (3.2), 405 (11.6), 404 (10.3), 403 (17), 370 (3.2), 307 (3.2), 269 (2.5), 268 (4.5), 267 (3.2), 239 (3.2), 238 (3.2), 237 (6.5) 235 (3.2), 223 (4.5), 222 (12.2), 221 (4.5), 220 (3.2), 210 (13), 209 (10.3), 208 (14), 199 (6.5), 198 (15.5), 197 (100), 195 (8.4), 194 (11.6), 190 (11), 184 (6.5), 183 (19.4), 182 (22.5), 181 (18.8), 180 (6.5), 171 (6.5), 170 (14.2), 168 (8.4), 167 (6.5), 158 (13), 144 (16.8). High-resolution mass spectra were obtained on a Consolidated Electrodynamics CEC High-Resolution Model 21-110.

SCHEME V



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Detection of an Acylable OH Group in Alstonisidine.—A solution of alstonisidine (3 mg) in pyridine (0.6 ml) and acetic anhydride (0.6 ml) was held 1 hr at 37° and 12 hr at 20°. The reaction mixture was freed of solvent under reduced pressure and the

(19) *Cf.* ref 16, p 84.

residue taken up in chloroform (15 ml). This solution was extracted with 14% aqueous ammonia (1 ml), dried ($MgSO_4$), and concentrated. The product was homogeneous on tlc[8] ($R_{alstonisidine}$ 1.1): micro-ir ν^{KBr} 1740, 1735 (ester C=O), no -OH; micro-nmr τ ($CDCl_3$) new 3H singlet at τ 7.92 (CH_3COO-): mass spectrum M^+ at m/e 714.

Detection of an Amino-Ketal Group in Alstonisidine.—Lithium aluminum hydride (15 mg) was added to a solution of alstonisidine (2 mg) in tetrahydrofuran (1 ml). The mixture was heated under reflux for 8 hr, cooled, and quenched with wet tetrahydrofuran. Addition of 25% sodium hydroxide (10 ml) dissolved the inorganic precipitate, and the alkaline solution was then extracted with chloroform (10 ml). The chloroform extract was dried (K_2CO_3) and solvent removed. Tlc[8] showed no alstonisidine remaining. The product was treated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) at 65° for 2 hr and at 20° for 60 hr. Solvents were removed under reduced pressure at 45°, and the residue was dissolved in chloroform. The solution was then washed with 5% ammonia and dried (K_2CO_3). Removal of solvent gave an oily residue which solidified on trituration with methanol: micro-ir ν^{KBr} 1740 cm^{-1}; M^+ 772 (calcd for $C_{47}H_{56}N_4O_6$, 772).

Treatment of macralstonidine with lithium aluminum hydride under the same conditions returned pure starting material. Villalstoninetriol was prepared by the published method.[4]

Chromotropic Acid Tests.—These were performed essentially as described for macralstonidine,[12] except that 4 mg of alstonisidine was used, and the colors were developed by adding chromotropic acid (1 mg) and 12 *N* sulfuric acid (2 ml) to 1–2-ml cuts of distillate. Blanks developed no color under these conditions, and the reaction with macralstonidine was used as a standard.

Registry No.—**5**, 27141-90-8; **5** monoacetate, 27248-70-0.

Acknowledgments.—We are greatly indebted to Drs. Norbert Neuss and John Occolowitz, Lilly Research Laboratories, for the indispensable high-resolution mass spectrometry, and to Gloria Cook and Mr. Forrest MacKellar for other spectral data. The MS-902 mass spectrometer at the University of Michigan was purchased with funds provided by the National Science Foundation. We gratefully acknowledge a Faculty Research Fellowship and Grant from the Horace H. Rackham School of Graduate Studies, University of Michigan (to P. W. Le Q.), and the award of a NASA Fellowship (to J. M. C.).

Preparation and Properties of Steroidal 17,20- and 20,21-Acetonides Epimeric at C-20. III. Dioxolone Derivatives of α-Hydroxy Acids[1]

MARVIN L. LEWBART

Department of Medicine, Jefferson Medical College, Philadelphia, Pennsylvania 19107

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The common chromic anhydride–pyridine oxidation product of the 17,20α- and 17,20β-acetonido-21-ols **1a** and **1b** has been identified as the etienic acid acetonide (dioxolone) **2**. In a study of the reaction sequence involved in its formation, it was shown that the 20-methylene derivative **7** is not an intermediate, and that the 17,20-acetonido-21-aldehydes are the immediate precursors of **2**. A general method for the synthesis of dioxolones from α-hydroxy acids, using acetone–perchloric acid, was devised, and the preparation of eight examples from 17-hydroxyetianic and 20-hydroxypregnanoic acids in the 11-oxygenated Δ^4-3-keto series is described. All dioxolones are readily cleaved by dilute alkali, but are considerably more resistant to hydrolysis with 60% acetic acid at room temperature than isopropylidene derivatives of the corresponding glycols. Dioxolones from 17-hydroxyetianic and 17-deoxy- and 17-hydroxy-20-hydroxypregnanoic acids can be distinguished by their ir and nmr spectra.

We recently reported[2] that prolonged reaction of the 17,20α- and 17,20β-acetonido-21-ols **1a** and **1b** (Scheme I) with chromic anhydride in pyridine gives rise to not only the respective 21-aldehydes and 21-oic acids, but also a common neutral product with the empirical formula $C_{23}H_{30}O_5$. This substance has been identified as the etienic acid acetonide (dioxolone) **2**. In addition to presenting evidence for this structure, this paper includes a general procedure for the independent preparation of dioxolones not only from 17-hydroxyetianic acids, but also from homologous 20-hydroxypregnan-21-oic acids epimeric at C-20. Finally, some of the properties of the new derivatives will be discussed.

Structural assignment of the etiodioxolone **2** was based on the following considerations: (a) infrared spectroscopy indicated a new carbonyl band at 1782 cm^{-1} and retention of the isopropylidene group as evidenced by a characteristic doublet at 1385 and 1377 cm^{-1}; (b) its mass spectrum displayed a prominent molecular $(M)^+$ ion, m/e 386, as well as the m/e 328 ion, representing M less the elements of acetone [However, ions such as M − 15 (CH_3) and M − 15 − 60 (HOAc), which have been observed in both 17,20- and 20,21-acetonides, were not seen. Their absence is understandable in view of the greater complexity and, accordingly, lessened stability of the dioxolone ring.];[3] and (c) treatment of **2** with methanolic sodium hydroxide or methanolic hydrogen chloride gave the methyl etienate **3**.

An investigation was made of the mechanism of formation of **2** from **1a** and **1b**. A plausible sequence of reactions at C-21 would be $CH_2OH \rightarrow CHO \rightarrow COOH$, followed by decarboxylation. Subsequent oxidation of the resulting 20-methylene group to a carbonyl would afford the dioxolone **2**. Accordingly, the hypothetical 20-methylene intermediate **7** was prepared as follows. Treatment of the methyl etienate **4** with pyrrolidine in hot methanol[4] gave the crystalline 3-enamine. Its lithium aluminum hydride reduction, followed by buffered hydrolysis of the product, afforded the glycol **6** which was characterized by periodic acid oxidation to

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(2) M. L. Lewbart and J. J. Schneider, *J. Org. Chem.*, **34**, 3513 (1969).

(3) A detailed study of the mass spectral characteristics of a number of cyclic derivatives of the steroid side chain, including acetonides and dioxolones, will be presented at a later date.

(4) J. L. Johnson, M. E. Herr, J. C. Babcock, A. E. Fonken, J. E. Stafford, and F. W. Heyl, *J. Amer. Chem. Soc.*, **78**, 430 (1956).