A helenine sample, analyzed in the ultracentrifuge in the absence of glycerol, showed six components to be present. During electrophoresis, however, 90% of the material migrated as a single boundary. These data indicate that even though the components are of varying molecular weights, the majority of these possess a similar charge density.

Furthermore, as seen in Fig. 2A, most of the disintegration noted during ultracentrifugation was prevented by the use of glycerol. The glycerol made it possible to obtain a preparation that exhibited only two major peaks and one minor one.

The failure of the bacterial endotoxins and the lipopolysaccharide fraction from *P. funiculosum* to produce a helenine-like response in the assay suggests that helenine is different from these compounds. The low hexose content of our purified nucleoprotein fraction would also support this view.

Acknowledgment.—We wish to thank Miss Verda Powell and Mr. John Ruscica for many of the electrophoresis and ultracentrifuge determinations. We would also like to acknowledge the excellent technical assistance of Mrs. Elizabeth Hagan.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE RICE INSTITUTE, HOUSTON, TEX.]

Observations on the Structure of Oxonitine[1]

BY RICHARD B. TURNER, J. P. JESCHKE AND M. S. GIBSON

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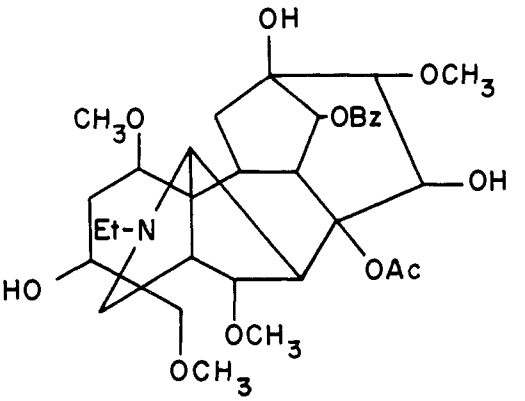
Conclusive evidence has been obtained in support of the contention of Pelletier that oxonitine, an oxidation product of aconitine, is an N-formyl derivative (IV). That the formyl group is not derived from the N-ethyl group of aconitine (for example, by oxidative cleavage of a vinylamine) is indicated by the results of radioactive tracer experiments. The N-acetyl derivative II has also been isolated as a minor product of the permanganate oxidation of aconitine, and cleavage of N-nitrosodesethylaconitine triacetate (VIII) by acetyl chloride and by phosgene has been demonstrated.

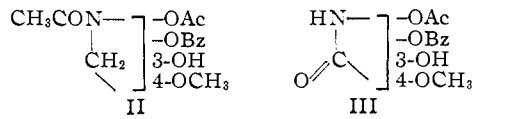
Early investigation of the Aconite alkaloid aconitine showed that this substance is a tertiary base of molecular formula $C_{34}H_{47}NO_{11}$ possessing an N-ethyl group, four methoxyl groups, three hydroxyl groups, one acetoxyl group and one benzoyloxy group.[2] The problem of skeletal structure proved exceedingly difficult, and little progress was made in its solution until 1956, when the structure of the closely related alkaloid, lycoctonine, was deduced by Przybylska and Marion from X-ray measurements.[3] The recent results of Wiesner, Büchi and their collaborators[4] now provide strong evidence in support of structure I for aconitine.

In the historical development of the aconitine work the permanganate oxidation product, oxonitine, occupied a position of considerable importance. Oxonitine possesses an amide linkage and gives negative results in the Herzig–Meyer determination indicating loss of the original N-ethyl group.[5] With the exception of this modification the functional groups of aconitine are present intact in the oxidation product. However, despite repeated investigation, the nature of the structural change involved in the formation of oxonitine has remained in doubt.



I

The uncertainty regarding the formulation of oxonitine has centered mainly on the two part structures II and III which correspond, respectively, to the molecular formulas $C_{34}H_{45}NO_{12}$ and $C_{32}H_{41}NO_{12}$. Although these alternatives should be easily distinguishable by elementary analyses,



considerable difficulty has been experienced with aconitine derivatives in the preparation of pure, solvent-free analytical samples.[6] Thus, Jacobs and Pelletier[7] report that oxonitine crystallized from chloroform contains a substantial amount of chlorine owing to tenacious retention of solvent. Acetic acid is also objectionable. While a series of samples giving carbon and hydrogen values corresponding closely to those calculated for II were

(1) This investigation was supported by a grant from the Robert A. Welch Foundation.

(2) *Cf.* K. Wiesner and Z. Valenta, "Progress in the Chemistry of Organic Natural Products," L. Zechmeister, editor, Vol. XVI, Springer-Verlag, Vienna, 1958, pp. 26–89; E. Stern, Chap. 37 in "The Alkaloids, Chemistry and Physiology," R. H. F. Manske and H. L. Holmes, editors, Vol. IV, Academic Press, Inc., New York, N. Y., 1954, pp. 275–333; T. A. Henry, "The Plant Alkaloids," J. A. Churchill, London, 1949, pp. 674–678.

(3) M. Przybylska and L. Marion, *Can. J. Chem.*, **34**, 185 (1956).

(4) K. Wiesner, M. Götz, D. L. Simmons, L. R. Fowler, F. W. Bachelor, R. F. C. Brown and G. Büchi, *Tetrahedron Letters*, No. 2, 15 (1959); K. Wiesner, F. Bickelhaupt, D. R. Babin and M. Götz, *ibid.*, No. 3, 11 (1959); K. Wiesner, D. L. Simmons and L. R. Fowler, *ibid.*, No. 18, 1 (1959); see also M. Przybylska and L. Marion, *Can. J. Chem.*, **37**, 1116, 1843 (1959).

(5) W. A. Jacobs and R. C. Elderfield, THIS JOURNAL, **58**, 1059 (1936).

(6) *Cf.* E. Späth and Fr. Galinovsky, *Ber.*, **63**, 2994 (1930).

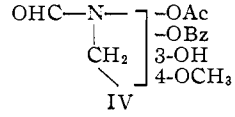
(7) W. A. Jacobs and S. W. Pelletier, THIS JOURNAL, **76**, 4049 (1954).

obtained by the use of ethanol, benzene, acetone or pyridine, the purity of these specimens was questioned in a subsequent communication.[8]

Evidence that has been interpreted in favor of structure III is as follows. Majima and Suginome[9] have reported oxonitine as an oxidation product of mesaconitine, which possesses an N-methyl rather than an N-ethyl group. More recently Schneider[10] subjected oxonitine to lithium aluminum hydride reduction and isolated an amorphous base, which failed to yield crystalline salts, but which, after treatment with ethyl iodide, afforded (in unspecified yield) a crystalline hydrochloride identical with aconine hydrochloride (*cf.* XIIa) obtained by hydrolysis of aconitine. The infrared absorption spectrum (KBr) of oxonitine was recorded by Schneider with the assignments: 2.87 (OH), 2.91 (NH),[11] 5.83 (ester carbonyl), 5.99 (6-ring lactam), 6.08 μ (C=C).

Assignment of the band at 6.08 μ to a carbon–carbon double bond is in error. In our hands permanganate oxidation of aconitine affords crystalline material showing infrared absorption in potassium bromide similar to that reported by Schneider. However, repeated recrystallization yields a product that exhibits only one band in the 6 μ region at 5.99 μ. The impurity responsible for absorption at 6.08 μ was ultimately isolated from the mother liquors and identified as the N-acetyl derivative II. The major product, oxonitine, therefore cannot possess this structure. In this connection it should be noted that the isolation of II casts considerable doubt on the validity of Schneider's argument, since the possibility that the aconine hydrochloride obtained in his experiments may have been derived from this contaminant is not excluded.

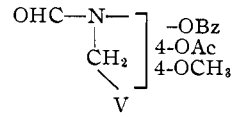
Very recently Pelletier has suggested that oxonitine in fact possesses the N-formyl structure IV.[12] The correctness of this proposal, which is based on the isolation of formic acid after drastic hydrolysis and on other observations, has now been conclusively established.



IV

In view of the problems associated with handling the extremely insoluble oxonitine, we have devoted most of our attention to the acetates of the series, since these derivatives can be purified without difficulty by chromatography. It may be mentioned, however, that our analytical data for oxonitine itself are in excellent agreement with the formula $C_{33}H_{43}NO_{12}$ required by part structure IV.

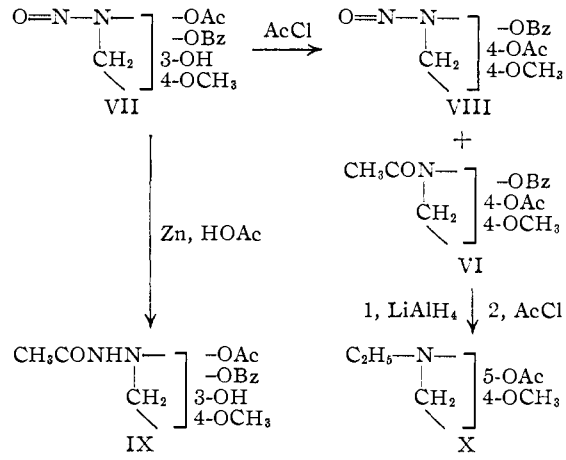
Oxidation of aconitine triacetate with potassium permanganate affords a mixture from which two pure, crystalline substances are readily separated on alumina. The major product, oxonitine triacetate (also obtained by direct acetylation of oxonitine), gives analytical values consistent with the formula $C_{39}H_{49}NO_{15}$ represented by part structure V. The substance absorbs in the infrared at 1669 cm.$^{-1}$



V

(5.99 μ) in potassium bromide and at 1660 cm.$^{-1}$ in chloroform solution as does oxonitine itself. The minor product (VI), $C_{40}H_{51}NO_{15}$, for which the name N-acetyldesethylaconitine triacetate is suggested, proved to be identical with the triacetate of II and showed the expected infrared absorption at 1640 cm.$^{-1}$ (6.10 μ) in potassium bromide and at 1627 cm.$^{-1}$ in chloroform solution. For purposes of comparison the infrared spectra of the following model compounds were measured in chloroform: N-acetylpiperidine (1628 cm.$^{-1}$), N-formylpiperidine (1661 cm.$^{-1}$) and piperidone (1658 cm.$^{-1}$).

The part structure assigned to VI was established as follows. Vigorous treatment of aconitine with nitrous acid results in cleavage of the N-ethyl group and formation of N-nitrosodesethylaconitine (VII).[13]



This product is converted in 52% yield into the corresponding triacetyl derivative VIII by the action of acetic anhydride and perchloric acid. Treatment with acetyl chloride, however, affords only 14% of VIII, the major product (57%) being N-acetyldesethylaconitine triacetate (VI) formed by acetolysis of the N-nitroso function. Experiments involving the use of radioactive acetyl chloride established the fact that three radioactive acetyl groups are introduced in VIII, whereas four such groups are incorporated in VI. An attempt to remove the nitroso group reductively with zinc and acetic acid gave the acetylhydrazine derivative IX as the only crystalline product.

Lithium aluminum hydride reduction of VI yields aconine (XIIa) as an oil devoid of amide absorption in the infrared. This substance gives a single spot, developed by the Dragendorff reagent,[14] on paper chromatography and is indistinguishable by this criterion and by infrared analysis from aco-

(8) W. A. Jacobs and S. W. Pelletier, *Chemistry & Industry*, 948 (1955).

(9) R. Majima and H. Suginome, *Ber.*, **58**, 2048 (1925); S Morio, *Ann.*, **476**, 181 (1929).

(10) W. Schneider, *Ber.*, **89**, 762 (1956).

(11) Interpretation of absorption in this region is hazardous in view of the presence of hydroxyl groups with obvious possibilities for hydrogen bonding. Carbonyl overtones may also be a complicating factor.

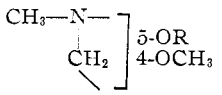
(12) S. W. Pelletier, private communication, August, 1959.

(13) A. Lawson, *J. Chem. Soc.*, 80 (1936).

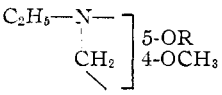
(14) K. Paech and M. V. Tracey in "Modern Methods of Plant Analysis," Vol. IV, Springer-Verlag, Berlin, 1955, p. 373.

nine derived by lithium aluminum hydride reduction of aconitine or of aconitine triacetate.[15] Acetylation of samples of aconine obtained by the various routes furnishes identical samples of crystalline aconine pentaacetate (X).[16]

When oxonitine triacetate (or oxonitine) is subjected to lithium aluminum hydride reduction, a crystalline base melting at 133–134° with resolidification and remelting at 215–217° is obtained in 76% yield. Elementary analyses indicate the formula $C_{24}H_{39}NO_9$, and the compound is formulated as mesaconine (XIa), which has hitherto been obtained only in amorphous form.[9] The infrared spectrum of this product is virtually indistinguishable from that of aconine, but the substance migrates more slowly than aconine on paper chromatography, the ratio of R_f values being 0.86.

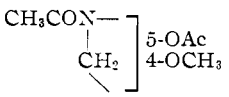


XIa, R = H
b, R = Ac



XIIa, R = H
b, R = Ac

Acetylation of XIa with acetyl chloride affords a *basic* pentaacetate (XIb), m.p. 235–237°, $[\alpha]_D$ −24.1°,[17] with analytical values in accord with the formula $C_{33}H_{47}NO_{14}$. Examination of the mother liquors in the infrared revealed the presence of material showing amide absorption at 1628 cm.$^{-1}$ corresponding to that expected for the acetolysis product XIII. The latter substance was obtained in



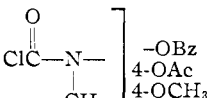
XIII

crystalline form in yields of about 20% by acetylation of XIa with acetic anhydride and perchloric acid, and its structure was established by an alternate preparation from N-acetyldesethylaconitine triacetate (VI) which involved hydrolysis of the ester functions and subsequent acetylation. The correlation of oxonitine with N-acetyldesethylaconitine (II), and hence also with aconine and aconitine, achieved in this way implies that skeletal rearrangements in the transformations described are unlikely.

The properties of XIb are very similar to those of aconine pentaacetate (XIIb), m.p. 248–249°, $[\alpha]_D$ −30.6°. Mixtures of the two compounds melt at intermediate temperatures, the pK's differ by only 0.2 unit,[18] and the X-ray powder diagrams are indistinguishable.[19] The infrared spectra are differentiated only by minor differences in the 1000–1150 cm.$^{-1}$ region. It is especially noteworthy that no absorption attributable to an NH group is detectable in the spectrum of XIb. The presence of an N-alkyl group is indicated by the results of Herzig–Meyer analysis, and there is hence no reasonable alternative to the formulation of XIb as a tertiary base.

Despite many points of similarity, XIb and aconine pentaacetate are nevertheless unquestionably different. Each substance on lithium aluminum hydride reduction yields a chromatographically homogeneous product, but reduction of a mixture of the two pentaacetates affords material that exhibits two spots on paper with an R_f ratio of 0.86.

Decisive evidence for the N-formyl structure assigned to oxonitine, and hence also for the N-methyl assignments in XIa and XIb, was derived as follows. N-Nitrosodesethylaconitine triacetate (VIII) was treated with phosgene under conditions similar to those employed for acetolysis with acetyl chloride. Chromatography of the crude product on alumina furnished a neutral (no titratable groups),[18] chlorine-containing substance, $C_{39}H_{48}NO_{15}Cl$. The infrared absorption spectrum of this compound showed no bands ascribable to a conventional amide linkage, but careful comparison with the spectrum of aconitine triacetate indicated a decrease in the intensity of C–H absorption of about 8% and an increase of intensity in the carbonyl region (unresolved) of about 20% for the new compound as compared with the aconitine ester.[18] Since the substance exhibits no discernible salt-like properties, it is assumed to possess the chloroformamide structure XIV.[20]



XIV

Treatment of the product XIV with formic acid–acetic anhydride resulted in smooth conversion into oxonitine triacetate (V), which proved to be identical in all respects with an authentic sample, and which differed from the known N-acetyldesethylaconitine triacetate (VI). Direct formolysis of N-nitrosodesethylaconitine triacetate (VIII) was also attempted, but the yield of V as judged by the intensity of the N-formyl band at 1661 cm.$^{-1}$ was poor.

One further piece of evidence remains to be considered. In connection with experiments already described it has been noted that treatment of N-nitrosodesethylaconitine (VII) with carboxyl-labeled (C^{14}) acetyl chloride yields N-acetyldesethylaconitine triacetate (VI) containing four radioactive acetyl groups. Reduction of the latter product with lithium aluminum hydride followed by acetylation with inert acetyl chloride furnishes aconine pentaacetate (XIIb) labeled in the ethyl group at the position adjacent to nitrogen. Permanganate oxidation of this material gives oxonine pentaacetate (XV), also obtainable from oxonitine by saponification and reacetylation,[21] in which the

(15) In our experience neither amorphous aconine nor its crystalline hydrochloride shows any absorption in the carbonyl region of the infrared; W. Schneider, *Ber.*, **89**, 768 (1956), reports an intense band at 5.85 μ in both substances.

(16) R. Majima and K. Tamura, *Ann.*, **526**, 116 (1936).

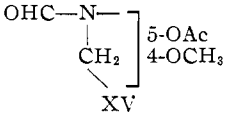
(17) Mesaconine pentaacetate is reported to melt at 228–229°, $[\alpha]_D$ −19.1° (see ref. 9).

(18) We are indebted to Dr. H. E. Boaz, Eli Lilly and Co., Indianapolis, Ind., for this result.

(19) Obtained through the courtesy of Prof. W. O. Milligan.

(20) The absence of amide absorption in the 1630 cm.$^{-1}$ region is attributed to the presence of an adjacent chlorine atom. Urethans, for example, absorb between 1736 and 1700 cm.$^{-1}$; see L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 1st edition, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 190.

(21) K. Tamura, *Ann.*, **533**, 183 (1938).



XV

residual radioactivity amounted to only 6% of that originally present in the aconine pentaacetate. It is therefore clear that the formyl group does not arise from the N-ethyl function, but that it is derived from an external source. This result is consistent with the observation of Pelletier[12] that the yield of oxonitine is markedly increased when aconitine is subjected to permanganate oxidation in the presence of methanol.

Experimental[22]

Preparation of Oxonitine (IV).[23]—A recrystallized sample (10.0 g.) of aconitine, m.p. 201–203°, $\nu_{max}^{CHCl_3}$ 3520, 1720 cm.$^{-1}$, in 400 ml. of acetone containing 1 ml. of acetic acid was treated at room temperature with 1.8 g. of potassium permanganate, added in portions with stirring. After disappearance of the permanganate color an additional 1 ml. of acetic acid was added followed by a further 1.8 g. of potassium permanganate. In all 7.2 g. of permanganate was employed in the oxidation. The reaction mixture was allowed to stand at room temperature for 24 hours, at the end of which time the manganese dioxide–oxonitine precipitate was removed by filtration and resuspended in ice-water. The manganese dioxide was then reduced with sulfur dioxide, and the residual oxonitine was filtered and repeatedly recrystallized from acetic acid–acetone. The yields of pure oxonitine, m.p. 284–286°, $[\alpha]^{28}D$ −45.2°, obtained in several experiments ranged from 1.5 to 2.5 g. The purified product possessed the spectral characteristics: ν_{max}^{KBr} 3480, 1706, 1670 cm.$^{-1}$; $\nu_{max}^{CHCl_3}$ 3510, 1717, 1658 cm.$^{-1}$.

Anal. Calcd. for $C_{33}H_{43}NO_{12}$: C, 61.38; H, 6.71; N, 2.17; O, 29.74. Found: C, 61.44, 61.49, 61.41; H, 6.88, 6.79, 6.77; N, 2.27, 2.33; O, 30.00, 29.94.

Preparation of N-Acetyldesethylaconitine (II).—The mother liquors remaining from the purification of oxonitine were concentrated under reduced pressure, and the residual material was crystallized repeatedly from chloroform–ethyl acetate and from aqueous methanol. A small sample of N-acetyldesethylaconitine was ultimately obtained, m.p. 274°, $[\alpha]_{5460}^{30}$ −52.4°; ν_{max}^{KBr} 3460, 1707, 1640 cm.$^{-1}$; $\nu_{max}^{CHCl_3}$ 3512, 1715, 1622 cm.$^{-1}$.

Anal. Calcd. for $C_{34}H_{45}NO_{12}$: C, 61.90; H, 6.87; N, 2.12; O, 29.11. Found: C, 62.05; H, 7.02; N, 2.35; O, 29.14.

Preparation of Aconitine Triacetate.[24] **(A).**—Aconitine (4.0 g.) was allowed to react with 60 ml. of acetyl chloride for 8 days at room temperature. The excess acetyl chloride was removed under reduced pressure, and the residue, after washing with dilute sodium bicarbonate solution, was chromatographed on alumina. Elution with benzene containing 10% ethyl acetate furnished 4.25 g. of aconitine triacetate, m.p. 211–212°. Several recrystallizations from ethyl acetate–petroleum ether gave material melting at 217–218°.

(B).—Five grams of aconitine was dissolved in 25 ml. of ice-cold acetic anhydride, and 1.4 ml. of 60% perchloric acid was then added dropwise with ice cooling. After 2 hours at 0° the product was isolated and chromatographed on alumina. Aconitine triacetate, 5.74 g., m.p. 216–217°, was obtained by this procedure. Recrystallization from ethyl acetate–petroleum ether afforded a sample, m.p. 218–219°, $[\alpha]^{30}D$ + 5.1°, $\nu_{max}^{CHCl_3}$ 1727 cm.$^{-1}$.

Anal. Calcd. for $C_{40}H_{53}NO_{14}$: C, 62.24; H, 6.92; N, 1.82. Found: C, 62.13; H, 7.31; N, 1.93.

Acetylation with C^{14}-labeled acetic anhydride gave a product containing 2.85 radioactive acetyl groups (theory 3.00).

Permanganate Oxidation of Aconitine Triacetate.—Potassium permanganate (4.5 g.) and acetic acid (4.0 ml.) were added in small portions to a stirred solution of 6.4 g. of aconitine triacetate in 500 ml. of acetone. After the permanganate color had disappeared, the solution was filtered, and the solvent was removed under reduced pressure. Chromatography of the residue on alumina gave 0.7 g. of unchanged starting material and 3.6 g. of **oxonitine triacetate (V)**, m.p. 257–258°, $[\alpha]_{5460}^{30}$ −65°, $\nu_{max}^{CHCl_3}$ 1729, 1660 cm.$^{-1}$, identical with a sample prepared by direct acetylation of oxonitine.[25] When the oxidation was carried out on the radioactive aconitine triacetate of the preceding experiment, oxonitine triacetate containing 2.96 radioactive acetyl groups (theory 3.00) was obtained.

Anal. Calcd. for $C_{39}H_{49}NO_{15}$: C, 60.69; H, 6.40; N, 1.82; O, 31.10; OCH_3, 16.09; N-alkyl, none. Found: C, 61.05; H, 6.54; N, 2.15; O, 31.55; OCH_3, 15.78; N-alkyl, none.[26]

The final fractions from chromatography of the crude oxidation product obtained in this experiment gave 0.1 g. of **N-acetyldesethylaconitine triacetate (VI)**, m.p. 183–184°, $[\alpha]^{26}D$ −57.8°; $\nu_{max}^{CHCl_3}$ 1738, 1627 cm.$^{-1}$. The identity of this product with a sample prepared by direct acetylation of II, and with material obtained as indicated in the following paragraphs, was established by conventional methods.

Preparation of N-Nitrosodesethylaconitine (VII).—The reaction of aconitine with nitrous acid was carried out according to the procedure of Lawson.[13] The product melted at 287–289°, $[\alpha]^{26}D$ −77°, ν_{max}^{KBr} 3500, 1710 cm.$^{-1}$, and gave a positive Liebermann test.

Anal. Calcd. for $C_{32}H_{42}N_2O_{12}$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.27; H, 6.17; N, 4.26.

Reaction of N-Nitrosodesethylaconitine (VII) with Acetyl Chloride.—The nitroso derivative (1.6 g.) was suspended in 20 ml. of acetyl chloride, and the mixture was stirred until solution was complete. After standing at room temperature for 8 days, the excess acetyl chloride was removed under reduced pressure, and the residue was taken up in chloroform. The chloroform solution was then washed with dilute sodium bicarbonate and water and was dried over anhydrous sodium sulfate. Removal of the solvent gave crude material that was chromatographed on alumina. Elution with benzene–ethyl acetate (90:10) gave 260 mg. of **N-nitrosodesethylaconitine triacetate (VIII)**, m.p. 262–263°, $[\alpha]_{5460}^{25}$ −70°, $\nu_{max}^{CHCl_3}$ 1733 cm.$^{-1}$.

Anal. Calcd. for $C_{38}H_{48}N_2O_{15}$: C, 59.06; H, 6.26; N, 3.63. Found: C, 59.15, 59.00; H, 6.36, 6.51; N, 3.63; 3.33.

When radioactive acetyl chloride was employed, a sample of VIII was obtained which possessed 2.82 radioactive acetyl groups (theory 3.00).

Elution with benzene–ethyl acetate (80:20) afforded 1.1 g. of **N-acetyldesethylaconitine triacetate (VI)**, m.p. 186–188°, $[\alpha]^{29}D$ −55.4°; ν_{max}^{KBr} 1733, 1650 cm.$^{-1}$; $\nu_{max}^{CHCl_3}$ 1728, 1627 cm.$^{-1}$. This product did not depress the melting

(22) Melting points were taken on a Fisher–Johns melting point stage. Infrared measurements were made on a Perkin–Elmer model 21 infrared spectrophotometer. Specific rotations were uniformly observed in chloroform solutions. Paper chromatograms were run by the ascending method in a 1-butanol–acetic acid–water system (volume ratio 4:1:5).

Analyses of C^{14}-labeled samples were carried out in toluene by the liquid phase scintillation technique. The comparison standard was cholesteryl acetate, m.p. 115–116°, prepared by treatment of cholesterol with a sample of the same reagent (acetyl chloride or acetic anhydride) used for acetylating the alkaloid derivatives. No correction was made for differences between quenching of the standard and that of the samples being analyzed. The background count was negligible in comparison with the counting rates of the compounds that were investigated.

(23) F. H. Carr, *J. Chem. Soc.*, **101**, 2241 (1912).

(24) W. R. Dunstan and F. H. Carr, *ibid.*, **67**, 459 (1895).

(25) H. Suginome, *Ann.*, **533**, 172 (1938), reports that oxonitine triacetate crystallizes from alcohol as a heptahydrate, m.p. 176–178°. K. Tamura, *ibid.*, **533**, 183 (1938), observed a melting point of 178° followed by resolidification and remelting at 235°. Tamura's sample, $[\alpha]D$ −50.8 (chloroform), on crystallization from ethyl acetate showed a single melting point of 233°. Material which we have obtained melts at 257° when crystallized rapidly from ethyl acetate and at 181–182° with solidification and remelting at about 245° when crystallized slowly from this solvent. The use of methylene chloride–petroleum ether gives only high melting material. The matter has not been investigated further.

(26) Methoxyl and N-alkyl determinations were obtained through the courtesy of Eli Lilly and Co., Indianapolis, Ind.

point of the sample obtained by oxidation of aconitine triacetate, and the infrared spectra of the two specimens were identical. The substance obtained when C^{14}-carbonyl-labeled acetyl chloride was used possessed 3.82 radioactive acetyl groups (theory 4.00).

Anal. Calcd. for $C_{40}H_{51}NO_{15}$: C, 61.13; H, 6.54; N, 1.78. Found: C, 61.39; H, 6.79; N, 1.92.

In some experiments later fractions (benzene-ethyl acetate, 50:50) furnished **N-acetyldesethylaconitine** diacetate, convertible into the triacetate by further acetylation. The diacetate melts at 249° and shows bands at 3525, 1723 and 1627 cm.$^{-1}$ in the infrared ($CHCl_3$ solution).

Anal. Calcd. for $C_{38}H_{49}NO_{14}$: C, 61.36; H, 6.64; N, 1.88. Found: C, 61.19; H, 6.86; N, 2.10.

Reduction of N-Nitrosodesethylaconitine (VII) with Zinc and Acetic Acid.—N-Nitrosodesethylaconitine (1.42 g.) in 150 ml. of acetic acid was stirred at 45–50° while 40 g. of zinc dust was added over a period of 12 hours. At the end of 24 hours the mixture was cooled, filtered, and concentrated under reduced pressure. The product was isolated by chloroform extraction and after crystallization from ethyl acetate gave 235 mg. of crude acetylamino derivative IX. Recrystallization from ethyl acetate–cyclohexane furnished a pure sample, m.p. 242°; $\nu_{max}^{CHCl_3}$ 3510, 1718, 1656 cm.$^{-1}$.

Anal. Calcd. for $C_{34}H_{46}N_2O_{12}$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.89; H, 7.06; N, 3.98.

Lithium Aluminum Hydride Reduction of C^{14}-Labeled N-Acetyldesethylaconitine Triacetate (VI).—N-Acetyldesethylaconitine triacetate (670 mg.), prepared by acetolysis of VII and containing four C^{14}-carbonyl labeled acetyl groups, was dissolved in tetrahydrofuran, and the resulting solution was added slowly to a refluxing solution of lithium aluminum hydride in tetrahydrofuran. After 8 hours the reaction mixture was chilled in ice, and the excess lithium aluminum hydride was destroyed by careful addition of water. The inorganic precipitate was removed by filtration and was extracted several times with hot chloroform. The filtrate and washings were then combined and evaporated to dryness. The residual material was taken up in 2 *N* hydrochloric acid, and the benzyl alcohol formed in the reduction was removed by chloroform extraction. The aqueous layer was neutralized and was then concentrated to dryness under reduced pressure, the temperature being held below 50°. Extraction of the dry residue with chloroform finally gave 332 mg. of crude, amorphous, ethyl-labeled aconine showing a strong OH band at 3520 cm.$^{-1}$ in the infrared, but no absorption in the carbonyl region.

The material showed a single spot, developed by the Dragendorff reagent, on paper chromatography and was chromatographically indistinguishable from a sample of aconine prepared by lithium aluminum hydride reduction of aconitine triacetate. The infrared spectra of the two samples were identical. A radioautogram of the paper strip showed a single spot at a position coincident with the Dragendorff spot. Radioactivity assay carried out without purification indicated the presence of 0.69 radioactive ethyl group (theory 1.00) in the crude aconine.

Acetylation of the radioactive aconine with inert acetyl chloride gave, after chromatography on alumina, a sample of **ethyl-labeled aconine pentaacetate**, m.p. 248–249°, containing 0.99 ± 0.03 (two determinations) radioactive ethyl group (theory 1.00). The substance did not depress the melting point of an authentic sample.

Preparation of Mesaconine (XIa).—Oxonitine triacetate (1.05 g.) in 39 ml. of tetrahydrofuran was added dropwise to a boiling solution of 2.5 g. of lithium aluminum hydride in 250 ml. of tetrahydrofuran (nitrogen atmosphere). The reaction mixture was heated under reflux for 8 hours and was then worked up by the procedure employed for the preparation of aconine. The product (XIa) crystallized from ethyl acetate; yield 500 mg., m.p. 131–132°. Recrystallization afforded a sample, m.p. 133–134°, that solidified above the melting point and remelted at 215–217°. The infrared spectrum was devoid of carbonyl absorption. The substance exhibited a single spot on paper chromatography and was clearly distinguishable from aconine by this method. The same product was obtained in 74% yield by lithium aluminum hydride reduction of oxonitine.

Anal. Calcd. for $C_{24}H_{39}NO_9$: C, 59.36; H, 8.10; N, 2.89. Found: C, 59.03; H, 8.21; N, 2.79.

Acetylation of Mesaconine (XIa).—Mesaconine (550 mg.) was dissolved in 15 ml. of acetyl chloride and allowed to stand at room temperature for 8 days. The excess reagent was then removed under reduced pressure, and the residue was taken up in chloroform, washed with dilute sodium bicarbonate, and dried over anhydrous sodium sulfate. Chromatography and crystallization from ethyl acetate furnished 580 mg. of mesaconine pentaacetate (XIb), m.p. 235–237°. The analytical sample melted at 236–237°, $[\alpha]^{23}D$ −24.1°, $\nu_{max}^{CHCl_3}$ 1734 cm.$^{-1}$, pK_a' 5.85 ± 0.05.[18]

Anal. Calcd. for $C_{34}H_{49}NO_{14}$: C, 58.69; H, 7.10; N, 2.01; O, 32.20; OCH_3, 17.84; NCH_3, 4.2. Found: C, 58.57, 58.38; H, 7.06, 6.92; N, 2.45, 2.32; O, 32.27, 31.60; OCH_3, 17.53, 17.25; N-alkyl, 4.0 (calculated as NCH_3).

Acetylation with radioactive active anhydride gave material possessing 4.92 radioactive acetyl groups (theory 5.00).

Acetylation of mesaconine (1.5 g.) with acetic anhydride (80 ml.) and 60% perchloric acid (2 ml.) for 2 hours at 0° gave in addition to mesoconine pentaacetate, 440 mg. of **N-acetyldesethylaconine pentaacetate (XIII)** which was isolated by chromatography. The material melted at 244–245°; $\nu_{max}^{CHCl_3}$ 1738, 1628 cm.$^{-1}$.

Anal. Calcd. for $C_{35}H_{49}NO_{15}$: C, 58.08; H, 6.82; N, 1.94; O, 33.16. Found: C, 58.39, 57.94; H, 6.66, 6.78; N, 2.28, 2.24; O, 32.84, 33.28.

A sample of XIII identical in all respects with that obtained in this experiment was prepared as indicated below.

Preparation of N-Acetyldesethylaconine Pentaacetate (XIII) from N-Nitrosodesethylaconitine (VII).—N-Nitrosodesethylaconitine (2.25 g.) was dissolved in 50 ml. of acetyl chloride, and the solution was allowed to stand at room temperature for 2 days. The excess acetyl chloride was then removed under reduced pressure, and the residual material was heated under reflux for 2 hours with 1.2 g. of sodium hydroxide in 40 ml. of ethanol and 50 ml. of water. At the end of this time the reaction mixture was neutralized with dilute hydrochloric acid and was evaporated to dryness. The total residue was acetylated with acetic anhydride–perchloric acid, and the product was finally chromatographed on alumina. N-Acetyldesethylaconine pentaacetate, 520 mg., m.p. 244–245°, was obtained which did not depress the melting point of material derived in the preceding experiment. The infrared spectra of the two samples were identical.

Preparation of Aconine Pentaacetate (XIIb).[16]—Amorphous aconine (2.0 g.) was acetylated with acetic anhydride and perchloric acid. Chromatography furnished 1.88 g. of aconine pentaacetate, m.p. 248–249°, $[\alpha]^{23}D$ −31.2°, $\nu_{max}^{CHCl_3}$ 1734 cm.$^{-1}$, pK_a' 6.05 ± 0.05.[18]

Anal. Calcd. for $C_{35}H_{51}NO_{14}$: C, 59.23; H, 7.24; N, 1.97; OCH_3, 17.49; NC_2H_5, 6.1. Found: C, 59.35; H, 7.23; N, 2.18; OCH_3, 17.00; NC_2H_5, 5.2.

Acetylation with radioactive acetyl chloride gave a sample containing 5.07 and 4.94 radioactive acetyl groups in two determinations (theory 5.00).

Lithium Aluminum Hydride Reduction of Aconine Pentaacetate (XIIb) and of Mesaconine Pentaacetate (XIb).—Reduction of 18 mg. of aconine pentaacetate with 0.4 g. of lithium aluminum hydride in 50 ml. of tetrahydrofuran furnished a product which showed an infrared spectrum identical with that of aconine and which was indistinguishable from aconine by paper chromatography. Reduction of a small sample of mesaconine pentaacetate gave material that was indistinguishable from mesaconine (XIa) by the same criteria. Both reduction products gave single spots on paper chromatography with an R_f ratio of 0.86.

A mixture of 10 mg. of aconine pentaacetate and 10 mg. of mesaconine pentaacetate was reduced similarly, and the product was chromatographed on paper. Two spots appeared with the characteristic R_f ratio of 0.86.

Reaction of N-Nitrosodesethylaconitine Triacetate (VIII) with Phosgene.—N-Nitrosodesethylaconitine triacetate (162 mg.) was sealed in a tube with 2 ml. of liquid phosgene, and the reaction mixture was allowed to stand at room temperature for 10 days. The tube was then opened and, after evaporation of the phosgene, the product was chromatographed on alumina. From the benzene–petroleum ether eluates there was obtained 120 mg. of material, which was crystallized from methylene chloride–petroleum ether;

yield 115 mg., m.p. 193–197° dec. Further recrystallization from the same solvent mixture gave the analytical sample, m.p. 195–197° dec., $\nu_{max}^{CH_2Cl_2}$ 1730 cm.$^{-1}$ (broad and unresolved). The substance showed no titratable groups in aqueous dimethylformamide solution and gave a negative Liebermann test.

Anal. Calcd. for $C_{39}H_{48}NO_{16}Cl$: C, 58.10; H, 6.00; N, 1.74; Cl, 4.40. Found: C, 57.95; H, 6.08; N, 1.91; Cl, 4.72.

Conversion of XIV into Oxonitine Triacetate (V).—The chloroformamide (XIV) of the previous experiment (29.5 mg.) was dissolved in 1 ml. of anhydrous formic acid and 1 ml. of acetic anhydride, and the mixture was heated overnight on the steam-bath. The solvent was removed under reduced pressure, and the product was taken up in methylene chloride–ether and washed with water and dilute sodium hydroxide. Crystallization from methylene chloride–petroleum ether afforded 20.3 mg. of material, m.p. 253–255°. Recrystallization from the same solvent pair raised the melting point to 256–257°, $[\alpha]_{5460}^{26}$ −64° (chloroform); $\nu_{max}^{CH_2Cl_2}$ 1730, 1661 cm.$^{-1}$. A mixed melting point determination with an authentic sample of oxonitine triacetate showed no depression, and the infrared spectra of the two specimens in methylene chloride, chloroform and carbon disulfide were identical.

Permanganate Oxidation of Ethyl-labeled Aconine Pentaacetate.—A sample of ethyl-labeled aconine pentaacetate (185 mg.), prepared as previously described, was oxidized with potassium permanganate in acetone by the method employed for oxidation of aconitine triacetate. Chromatography of the crude product on alumina afforded 42 mg. of unchanged starting material and 25 mg. of pure oxonine pentaacetate, m.p. 260–261°, $\nu_{max}^{CHCl_3}$ 1735, 1660 cm.$^{-1}$, identical with an authentic sample. Late fractions from the chromatogram showed infrared absorption at 1626 cm.$^{-1}$ and presumably contained N-acetylnoraconine pentaacetate. However, a pure sample of the latter compound could not be isolated.

Assay of the oxonine pentaacetate established the fact that only 6% of the activity originally present remained in the assay sample.

Anal. Calcd. for $C_{34}H_{47}NO_{15}$: C, 57.54; H, 6.68; N, 1.97; O, 33.82. Found: C, 57.71, 57.68; H, 6.43, 6.52; N, 2.26, 2.43; O, 33.54, 33.41.

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, SMITH, KLINE AND FRENCH LABORATORIES, PHILADELPHIA 1, PENNA.]

The Alkaloids of *Hortia arborea* Engl.[1]

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Seven bases were isolated from the bark of *Hortia arborea* Engl. Four of these are of the furoquinoline type; dictamnine, γ-fagarine, nor-γ-fagarine and skimmianine. The remaining three are of the quinazoline group: rutecarpine and two new alkaloids, hortiamine and hortiacine. Hortiamine, a red alkaloid, $C_{20}H_{17}N_3O_2$, yields 6-methoxy-1-oxo-1,2,3,4-tetrahydropyrid[3,4-b]indole and N-methylanthranilic acid when heated with ethanolic potassium hydroxide. The alkaloid was resynthesized through condensation of these degradation products. It is 10-methoxy-14-methyl-5-oxo-5,7,8,14-tetrahydroindolo[2,3-c]quinazo[3,2-a]pyridine. Hortiamine undergoes hydration in aqueous solvents to form a cyrstalline yellow compound, $C_{20}H_{19}N_3O_3$, which is 2-(*o*-methylaminobenzoyl)-6-methoxy-1-oxo-1,2,3,4-tetrahydropyrid[3,4-b]indole (6-methoxyrhetsinine). The hydrochloride of the alkaloid evolves gas upon fusion to yield a base, $C_{19}H_{18}N_2O_3$ identical with the second new alkaloid, hortiacine, which is shown to be 10-methoxyrutecarpine.

Several years ago our attention was directed to a Brazilian medicinal plant called "casca paratudo" which was said to possess excellent stimulant and stomachic properties. According to Hoehne,[3] "casca paratudo" is the bark of *Drimys winteri* Forst., a member of the family Magnoliaceae.

Samples of "casca paratudo" obtained from a commercial source[4] were found to contain alkaloids which, rather than being stimulant in action, were found to have sedative and hypotensive effects sufficient to warrant isolation and investigation of the individual bases. Initial fractionation studies showed that part of the alkaloids were of the furoquinoline type, typical of the family Rutaceae but not of the Magnoliaceae, and that botanical designation was therefore probably improper. Further investigation revealed that another Brazilian plant, *Hortia arborea* Engl., a member of the Rutaceae, also is called "casca paratudo." The plant is known for its bitter properties but is not reputed to have significant medicinal value. The materials used in the present study[5] were referred to the late botanist, Dr. J. G. Kuhlmann, who informed us that we were indeed dealing with *Hortia arborea* Engl.

The current work thus had fortuitous beginnings. Authentic *Drimys winteri* Forst.[6] was obtained eventually, but was found to possess neither alkaloids nor biological activity of note.

A concentrated ethanolic extract of milled trunk bark of *Hortia arborea* Engl. was partitioned between aqueous ammonia and chloroform. The chloroform layer was extracted with dilute hydrochloric acid, whereupon the alkaloids separated into two fractions: those whose salts remained in acid solution and those whose salts precipitated as yellow solid.

The acid-soluble alkaloids were liberated with aqueous ammonia, dissolved in benzene and precipitated as their hydrochlorides. The latter were dissolved in hot methanol, from which dictamnine (I) hydrochloride crystallized on cooling in 0.003% yield. Dictamnine was identified as its picrate[7] and by direct comparison with an authentic sample.[8]

The residual methanolic solution was evaporated to dryness and the bases regenerated. On stand-

(1) Presented, in part, at the 132nd Meeting of the American Chemical Society, New York City, N. Y., September, 1957, and at the International Congress for Pure and Applied Chemistry, Paris, July, 1957.

(2) Instituto de Quimica Agricola, Ministério da Agricultura, Rio de Janeiro, Brazil.

(3) F. C. Hoehne, "Plantas e Substâncias Vegetais Tóxicas e Medicinais," "Graphicors," São Paulo, Brazil, 1939.

(4) Flora Medicinal, Rio de Janeiro, Brazil.

(5) Specimens and barks derived from trees used in the present study have been deposited at the Herbarium of the Philadelphia Academy of Natural Sciences under numbers 859,232, 859,233 and 859,234.

(6) Specimen number 859,236 at the Herbarium of the Philadelphia Academy of Natural Sciences.

(7) Y. Asahina, T. Ohta and M. Inubuse, *Ber.*, **63**, 2045 (1930).

(8) We are grateful to Dr. J. R. Price of the C.S.I.R.O., Melbourne, Austr., for samples of dictamnine, γ-fagarine and skimmianine.