para methyl group, in agreement with the results in the 2-pentyl series,[4] the corresponding term for the α-methyl group shows an apparently real temperature dependence. The temperature dependence of this free energy of activation difference is reminiscent of the similar effect observed by Shiner on 2,3-dimethyl-2-chlorobutane[2] and may be connected with the relatively stable olefins or carbonium ions formed in both cases and with the possibility of plural reaction paths.

DEPARTMENT OF CHEMISTRY
THE RICE INSTITUTE EDWARD S. LEWIS
HOUSTON, TEXAS GALVIN M. COPPINGER

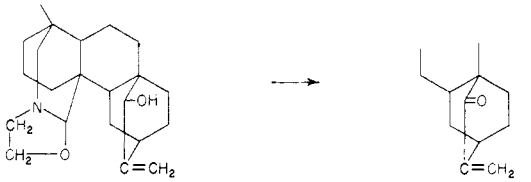
RECEIVED JUNE 17, 1954

THE ACONITE ALKALOIDS. XXVII. THE STRUCTURE OF ATISINE

Sir:

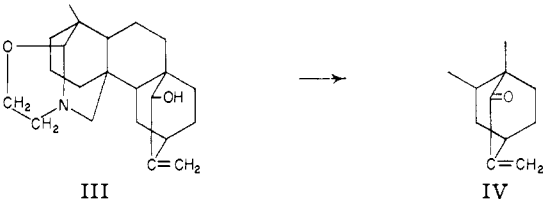
Recently we proposed a structure for atisine[1,2] based on the isolation of 6-ethyl-1-methylphenanthrene from the dehydrogenation of atisine,[3,4] oxoisoatisine[2] and tetrahydroatisine[2] as well as on other studies.[5,6] The formation of tetrahydro and diacyl derivatives suggested the presence of two centers of unsaturation and two hydroxyl groups in the molecule.[3] The diterpenoid structure first suggested[7] was modified and the double bonds placed so as to account for the marked differences in basicity of atisine (*pK* 12.2), isoatisine (*pK* 10) and dihydroatisine (*pK* 8, 2).[2,8] It now appears that a more normal diterpenoid structure better fits the available data. The fact that atisine shows only one C-methyl group[3] and that the lactam group of both oxoisoatisine and oxoisoatisine tricarboxylic acid is unusually resistant to hydrolysis[6] suggests one of the diterpene geminal methyl groups as the site of the lactam group. Wiesner, *et al.*, on the basis of the similarity of the chemistry of atisine and isoatisine to that of veatchine and garryine[9] have recently suggested structures I and III for atisine and isoatisine, though little supporting evidence was presented.[10] We wish to report two series of experiments which support the structures shown for atisine and its derivatives.

Treatment of atisine with chromium trioxide–pyridine complex[11] at 30° furnished an α,β-unsaturated ketone (II) in 60% yield, m.p. 102–103°, $[\alpha]^{31}D$ −27° (*c* 2.33 in chf.). Calcd. for $C_{22}H_{31}NO_2$: C, 77.37; H, 9.15. Found: C, 77.68, 77.60; H, 9.24, 9.32. The ultraviolet spectrum (EtOH) showed $\lambda_{max.}$ 228 mu, ϵ 9,100; $\lambda_{max.}$ 318 mu, ϵ 41; $\lambda_{max.}$ 342, ϵ 61. The infrared spectrum (chf.) showed bands at 3012, 1638 (strong) and 895 cm.$^{-1}$ (>C=CH$_2$); 1702 cm.$^{-1}$ (>C=O), *but the absence of any band attributable to a hydroxyl group.* III showed the absence of active hydrogen. Furthermore, atisine itself showed the presence of *only one active hydrogen.*[12] These data demonstrate that the $-NCH_2CH_2O-$ in atisine *must be present in a ring*, and not as a free $-NCH_2CH_2OH$ group as previously maintained.[2,7,13]



I II

That an oxide ring is also present in isoatisine (III) was shown by analogous oxidation of isoatisine with chromium trioxide–pyridine to the α,β-unsaturated ketone (IV), m.p. 159–163°, then 285–295° dec., $[\alpha]^{29}D$ −9.3° (*c* 1.89 in chf.). Found: C, 77.49; 77.50; H, 9.17, 9.34. The infrared spectrum (film from chf.) showed bands at 3077, 1631 (strong) and 884 cm.$^{-1}$ (>C=CH$_2$); 1710 cm.$^{-1}$ (>C=O), *but again the absence of any hydroxyl band in the 3400 cm.$^{-1}$ region.* The Tschugaeff–Zerewitinoff determination was negative.



III IV

Oxidation of atisine with potassium permanganate has given a lactam, the oxoatisine dicarboxylic acid (V).[2,6] Examination of the infrared absorption of its dimethyl ester (VI) reveals bands at 1715 (broad, $-CO_2Me$) and 1639 cm.$^{-1}$ (six-membered lactam) *but no band indicative of a hydroxyl group.*[14] The Tschugaeff-Zerewitinoff determination was negative.

A similar controlled oxidation of isoatisine (III) has furnished oxoisoatisine dicarboxylic acid (VII).[2,6] The infrared spectrum of its dimethyl ester (VIII) shows a band attributable to a hydroxyl group at 3380 cm.$^{-1}$, as well as bands at 1730 ($-CO_2Me$) and 1620 cm.$^{-1}$ (six-membered lactam). Furthermore, hydrogenation of oxoatisine dicarboxylic acid (V) furnished a product identical in all respects with oxoisoatisine dicarboxylic acid (VII) as shown by m.p., rotation, and infrared spectra. These results indicate that in the case of atisine oxidation proceeds

(1) E. S. Stern, "The Aconitum and Delphinium Alkaloids" in "The Alkaloids, Chemistry and Physiology," edited by R. H. F. Manske and H. L. Holmes, Vol. IV, Academic Press Inc., New York, N. Y., 1954, p. 275.

(2) W. A. Jacobs, *J. Org. Chem.*, **16**, 1593 (1951).

(3) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **143**, 589 (1942).

(4) C. F. Huebner and W. A. Jacobs, *ibid.*, **170**, 203 (1947).

(5) L. C. Craig and W. A. Jacobs, *ibid.*, **152**, 651 (1944).

(6) *Ibid.*, **170**, 515 (1947); **174**, 1001 (1948).

(7) L. C. Craig, L. Michaelis, S. Granick and W. A. Jacobs, *ibid.*, **154**, 293 (1944).

(8) O. E. Edwards and T. Singh, *Canad. J. Chem.*, **32**, 465 (1954).

(9) K. Wiesner, *et al.*, *ibid.*, **30**, 608 (1952); *Ber.*, **86**, 800 (1953); *Chem. and Ind.*, **132** (1954).

(10) K. Wiesner, personal communication, Feb. 16, 1954; M. F. Bartlett, Ph.D. Thesis Summary, Univ. of New Brunswick, May, 1954.

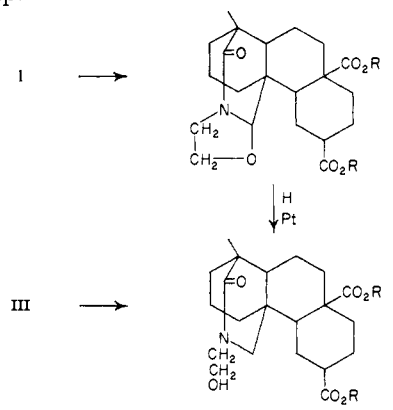
(11) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(12) This test was performed on undistilled atisine. Distillation apparently furnished altered material as shown by a different infrared spectrum from undistilled atisine[8] and by two active hydrogens in the Zerewitinoff test.[5,8]

(13) The ease of opening this ring to furnish $-NCH_2CH_2OH$ accounts for the ready formation from atisine of dihydroatisine on reduction, a diacetate on acylation, and the formation of glyoxal on treatment with lead tetraacetate. The latter reaction has been cited as proof of the existence of a preformed $-NCH_2CH_2OH$ group in atisine.[8]

(14) This experiment was suggested to us by Prof. K. Wiesner.[10]

without rupture of the oxide ring, while with isoatisine oxidation proceeds with rupture of the oxide ring, introduction of the lactam carbonyl at the site of rupture and the formation of a free $-NCH_2CH_2OH$ group.



THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH
NEW YORK 21, NEW YORK
S. W. PELLETIER
WALTER A. JACOBS

RECEIVED JULY 19, 1954

AMEBACIDAL ACTIVITY OF PUROMYCIN[1] IN THE GUINEA PIG

Sir:

In the course of the screening program being conducted in this laboratory for the evaluation of potential amebacidal agents, it has been observed that Puromycin (Lederle), an antibiotic form *Streptomyces albo-niger*, possesses significant activity against experimenally induced *Endamoeba histolytica* infections in the guinea pig. Thisco mpound has previously been reported to have significant activity against trypanosomiasis infections in experimental animals.[2,3] The structure of Puromycin has recently been elucidated.[4]

The test procedure for our amebiasis program has been described by Taylor and Greenberg.[5] All compounds are administered orally in solution, twice daily for five days. Simaroubidin (Merck) is used as the reference drug. At its minimum effective dosage of 2.5 mg./kg. of body weight, more than 98% of the infections are cured by it.

Puromycin (as the dihydrochloride) was initially tested at levels of 50 and 25 mg./kg. of body weight. At these levels the compound was highly effective against the induced amebic infections in the guinea pig. The drug has now been tested at lower levels, and the minimum effective dosage has been established at 6.25 mg. of the dihydrochloride per kg. of body weight, equivalent to 5.40 mg. of the free base. This compares with an oral LD_{50} for the dihydrochloride in non-inoculated guinea pigs of 600 mg./kg. of body weight (19/20 confidence limits), equivalent to 520 mg./kg. of the free base.

At 50 mg./kg., the highest oral dosage level employed to date therapeutically, there has been no evidence of drug toxicity. Several other antibiotics (Terramycin, Aureomycin, Chloromycetin, etc.) similarly tested produced weight loss and severe diarrhea. These toxic manifestations appeared following the fourth dose of the test compound. Diarrhea and weight loss due to amebic infection do not ordinarily appear until seven to nine days after intracecal injection of the parasites.

The amebacidal activity of seven antibiotics has previously been reported from this laboratory[5]; three additional ones have now been tested along with Puromycin. All three of the latter were ineffective at the dosages employed, *viz.*, erythromycin (Erythrocyn, Abbott), up to 50 mg./kg.; Magnamycin (Pfizer), up to 100 mg./kg.; and tetracycline (Tetracyn, Pfizer, Roerig), up to 50 mg./kg.

Analogs and degradation products of Puromycin are now being tested for their amebacidal activity.

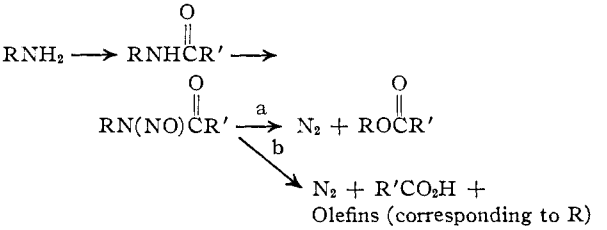
NATIONAL MICROBIOLOGICAL INSTITUTE
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
BETHESDA 14, MARYLAND
D. JANE TAYLOR
JOHN F. SHERMAN
HOWARD BOND

RECEIVED JULY 28, 1954

A NEW METHOD FOR THE DEAMINATION OF ALIPHATIC AMINES

Sir:

I wish to report a new method for the deamination of aliphatic amines. The steps involved are: acylation of the amine, nitrosation of the amide, and thermal elimination of nitrogen from the resulting N-alkyl-N-nitrosoamide.[1]



The two reaction paths account quantitatively for the nitrosoamide used. The esters from path *a* are relatively free of isomers and obtained in high yield, in marked contrast to the products from the classical deamination procedure with nitrous acid.[2]

Standard procedures were used for the acylations and some of the nitrosations. A more convenient method for nitrosating the amide (1 mole) was developed using nitrogen tetroxide[3] (1.5 moles) in the presence of anhydrous sodium acetate (3 moles) at

(1) In earlier publications, this compound was referred to as Achromycin, the name now applied by the Lederle Laboratories to their brand of tetracycline.

(2) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos and J. H. Williams, *Antibiotics and Chemotherapy*, **2**, 409 (1952).

(3) R. I. Hewitt, W. S. Wallace, A. R. Gumble, E. R. Gill and J. H. Williams, *Am. J. Trop. Med. Hyg.*, **2**, 254 (1953).

(4) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953).

(5) D. J. Taylor and J. Greenberg, *Am. J. Hyg.*, **56**, 58 (1952).

(1) Previous studies in this field have been concerned largely with the conversion of nitrosoamides into diazoalkanes. M. F. Chancel (*Bull. soc. chim. France*, (3) **13**, 125 (1895)) and H. v. Pechmann (*Ber.*, **31**, 2640 (1898)), however, have noted the instability of the nitrosoamides and the formation of esters from their decomposition; other than these observations, no pertinent work has been reported.

(2) For the reaction of nitrous acid with *n*-butylamine, F. C. Whitmore and D. P. Langlois (THIS JOURNAL, **54**, 3441 (1932)) report *n*-butanol (25%), *sec*-butanol (13%), 1-chlorobutane (5%), 2-chlorobutane (2%), and butenes (37%).

(3) Standard solutions (1–2 *M* in N_2O_4) were prepared by passing NO_2 into carbon tetrachloride or acetic acid at 0°.