COMMUNICATIONS TO THE EDITOR

A NEW CYCLIZATION REACTION[1]

Sir:

We wish to report that α-(2-biphenylyl)-acetonitrile as well as certain α-alkyl- and α-aroyl derivatives in the presence of concentrated sulfuric acid will undergo isomerization to yield phenanthrylamines. After three hours at 0°, 2-biphenylylacetonitrile afforded an 85% yield of 9-phenanthrylamine, m.p. 134–136° (lit.[2] 137.5–138°).

From α-(2-biphenylyl)-butyronitrile[3] and the homologous valeronitrile,[4] 10-ethyl-9-phenanthrylamine (m.p. 117.5–119.5°; benzamide, m.p. 241–243°) and 10-propyl-9-phenanthrylamine (m.p. 101–102.5°; benzamide, m.p. 220–221.5°) were obtained in 80–85% yield.

This new cyclization has been applied to α-(*o*-methoxybenzoyl)-α-(2-biphenylyl)-acetonitrile (obtained in a manner analogous to that used in preparing the isomer[5]) which in the presence of sulfuric acid yielded an amine (m.p. 175–176°) having the composition expected for 10-(*o*-methoxybenzoyl)-9-phenanthrylamine. This, as well as the other phenanthrylamines above, gave the characteristic diazonium coupling test with sodium β-naphtholate. On the basis of this same test, the compound obtained by the sulfuric acid cyclization of α-(*p*-methoxybenzoyl)-α-(2-biphenylyl)-acetonitrile, and originally described by Bradsher and Kittila[5] as 9-(*p*-methoxyphenyl)-10-phenanthramide is actually 10-(*p*-methoxybenzoyl)-9-phenanthrylamine.

(1) This investigation was supported by a research grant (C-1743) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) L. F. Fieser, R. P. Jacobsen and C. C. Price, THIS JOURNAL, **58**, 2163 (1936).

(3) C. K. Bradsher and W. J. Jackson, Jr., *ibid.*, **73**, 3235 (1951).

(4) C. K. Bradsher and W. J. Jackson, Jr., unpublished work.

(5) C. K. Bradsher and R. S. Kittila, THIS JOURNAL, **72**, 277 (1950).

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2-METHYLADENINE, AN HYDROLYSIS PRODUCT OF PSEUDOVITAMIN B_{12d}

Sir:

Holdsworth, *et al.*,[1] using paper ionophoresis with bioautographic techniques, demonstrated two microbiologically active components in crystalline pseudovitamin B_{12}.[2] This material had satisfied the accepted criteria of chemical homogeneity, including paper chromatographic study in a variety of solvent systems. Quantitative electrophoretic analysis of the same preparation examined by Holdsworth, *et al.*,[1] yielded 90% pseudovitamin B_{12}, 9% of a faster moving, microbiologically active, red pigment, and 1% of a very rapidly moving, microbiologically inactive, red pigment. The separation was conducted on cellulose columns in 0.1 *M* acetic acid containing a trace of NaCN with a potential of 200 v. and 0.002 amp. The microbiologically active contaminant readily crystallized from aqueous acetone and is referred to as pseudovitamin B_{12d}.[3] *Anal.*[4]: C, 52.68; H, 6.64; N, 16.90; Co, 4.36; P, 2.29. The composition is approximately that of pseudovitamin B_{12}. *Anal.* (before electrophoresis): C, 52.06; H, 6.60; N, 16.37; Co, 4.40; P, 2.31. (After electrophoresis): C, 52.35; H, 6.68; N, 17.15; Co, 4.41; P, 2.32.

Pseudovitamins B_{12} and B_{12d} have identical absorption spectra[5] in water with maxima at 278, 308, 320, 361, 518 and 548–50 mμ with $E^{1\%}_{1\,cm.}$ of 130, 62, 60, 204, 54.5, 57.5, respectively.

Pseudovitamin B_{12} is 0.7 and pseudovitamin B_{12d} 0.3 as active as vitamin B_{12} in growth assay with *L. leichmannii*.[6]

Pseudovitamin B_{12} differs from vitamin B_{12} in containing adenine[7] in nucleotide linkage in place of 5,6-dimethylbenzimidazole. The corresponding base in pseudovitamin B_{12d} is 2-methyladenine.

Pseudovitamin B_{12d} (4.47 mg.) in 4 ml. of 1 *M* HCl was heated in a sealed tube for 4 hours at 100°. The hydrolysate was percolated through a column (0.9 cm. × 5.5 cm.) of Amberlite 1R-120. The resin was washed with water (25 ml.), and then eluted with 2 *M* NH_4OH (30 ml.). The eluate absorbed ultraviolet light (max. 270 mμ). It was concentrated and examined for purines by paper chromatography, using successively the three systems: *n*-butanol–acetic acid–water (R_f 0.35), isoamyl alcohol–5% disodium phosphate (R_f 0.38), and 65% aqueous isopropyl alcohol–2 *M* HCl (R_f 0.64).[8] The ultraviolet absorption properties of the resin eluate as well as the eluates from the three paper chromatograms were identical with those of 2-methyladenine[9] (maxima at 265 mμ in 0.1 *M* HCl and 270 mμ at *p*H 11). Comparative and mixed paper chromatograms with 2-methyladenine yielded identical R_f values in the three solvent systems.

The eluate from the 65% aqueous isopropyl alcohol–2 *M* HCl chromatogram, containing by spectrographic analysis 275γ of the purine base was concentrated and a crystalline picrate prepared

(1) E. S. Holdsworth, J. E. Ford, S. K. Kon and J. W. G. Porter, *Nature*, **171**, 148 (1953).

(2) J. J. Pfiffner, D. G. Calkins, *et al.*, Abst. of 120th Meeting of Am. Chem. Soc., New York, 1951, 23 C.

(3) This pigment was found by Holdsworth, *et al.*,[1] to have the same mobility and microbiological growth activity as the main ionophoretic component of their Factor A from calf faeces.

(4) By Mr. William Saschek, University of Chicago.

(5) By Dr. J. M. Vandenbelt and associates.

(6) By Dr. O. D. Bird and associates.

(7) H. W. Dion, D. G. Calkins and J. J. Pfiffner, THIS JOURNAL, **74**, 1108 (1952); *Federation Proc.*, **11**, 269 (1952). The purine base has since been isolated as its crystalline picrate and identity with adenine picrate established by direct comparison of the X-ray powder diagrams.

(8) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

(9) J. Baddiley, B. Lythgoe, D. McNeil and A. R. Todd, *J. Chem. Soc.*, **383** (1943); J. Baddiley, B. Lythgoe and A. R. Todd, 318 (1944). A sample of 2-methyladenine was supplied by Dr. E. F. Godefroi and Dr. E. L. Wittle.

in the usual manner. Direct comparison of the X-ray powder diagrams[10] of the recrystallized picrate and of 2-methyladenine picrate proved their identity.

Ribose was identified in the filtrate from the resin column and 1-aminopropanol-2 in the ammoniacal eluate using paper chromatographic methods.[7,11]

The occurrence of 2-methyladenine in nature has not been observed previously. Many of the solvent systems employed in current paper chromatographic surveys of nucleic acid composition fail to differentiate 2-methyladenine from adenine.

(10) By Mr. R. B. Scott.

(11) S. M. Partridge, *Biochem. J.*, **42**, 238 (1945); *Nature*, **164**, 443 (1949); E. Chargaff, *et al.*, *J. Biol. Chem.*, **175**, 70 (1948).

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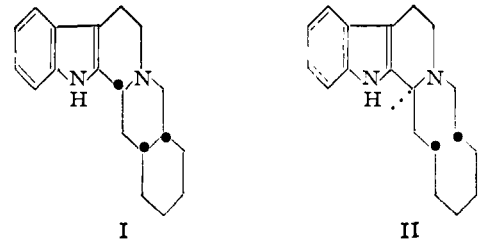
THE STEREOSPECIFIC SYNTHESIS OF *dl*-ALLOYOHIMBANE AND *dl*-3-EPIALLOYOHIMBANE

Sir:

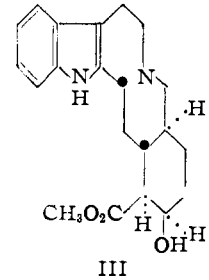
Three of the four possible steric arrangements of the ring system present in the yohimbe alkaloids have so far been found in nature: they are the ring systems present in yohimbane,[1] ψ-yohimbone[2] and alloyohimbane.[3]

In connection with the problem of the stereochemistry of the yohimbe alkaloids and closely related substances, such as reserpine,[4] it is important (a) to establish rigidly the stereochemistry of these systems[5]; (b) to synthesize the missing fourth isomer, 3-epialloyohimbane. Both of these goals have now been reached: *cis*-β-hydrindanone[6a] was prepared by cyclization of *cis*-cyclohexane-1,2-diacetic acid,[6b] itself made by ozonolysis of oxalyl β-decalone which was in turn prepared from crystalline *cis*-β-decalol, m.p. 105°,[6b] and *cis*-β-decalone. Subsequent steps were designed so as not to affect the *cis* junction established in the hydrindanone. Opening of the cyclic ketone by treatment with perbenzoic acid led to the lactone of *cis*-2-hydroxymethylcyclohexaneacetic acid, b.p. 115–120° (4 mm.). Calcd. for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 70.37; H, 8.90. This could be opened with hydrogen bromide in alcohol to ethyl *cis*-2-bromomethylcyclohexaneacetate, b.p. 100–106° (1 mm.). Calcd. for $C_{11}H_{19}O_2Br$: C, 50.20; H, 7.28. Found: C, 50.45; H, 7.45. Heating the bromoester in dimethylformamide solution with tryptamine gave, after chromatography, *cis*-N-3-indolylethyl-octahydro-3-isoquinolone, m.p. 171–172°. Calcd. for $C_{19}H_{24}ON_2$: C, 76.99; H, 8.16. Found: C, 76.87; H, 8.02. Cyclization of the lactam with phosphorus oxychloride gave an unstable vinylamine which was immediately reduced catalytically to the saturated base (I), m.p. 143.5–144°. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63. Found: C, 81.24; H, 8.51. Mixed melting point determination and comparison of infrared spectra demonstrated the identity of this base with *dl*-alloyohimbane.[7] Reduction of the vinylamine with sodium and alcohol in liquid ammonia solution gave the C_3 epimer (II) of *dl*-alloyohimbane, m.p. 185–186°. Found: C, 81.66; H, 8.65.

This synthesis, incidentally, demonstrates that alloyohimbane has a *cis*-decahydroisoquinoline system and the assumed stereochemistry shown in III[5] is therefore established for yohimbine.[8]



I II



III

(5) The correct stereochemistry of the yohimbe alkaloids was derived by one of us (see B. Witkop and S. Goodwin, THIS JOURNAL, **75**, 3371 (1953), footnote 6) and by M.-M. Janot, R. Goutarel, A. Le Hir, M. Amin, and V. Prelog, *Bull. soc. chim.*, 1085 (1952), on the basis of the existence in yohimbine of a *trans*-decahydroisoquinoline system. This assumption was however not rigidly established until the completion of the work described in this Communication, (*cf.* footnote 8), as it rested *either* on high temperature base degradations leading to octahydroisoquinolines with the double bond at, or adjacent to, the ring junction (B. Witkop, THIS JOURNAL, **71**, 2559 (1949)) *or* on an assumed, but unknown, course of the catalytic hydrogenation of sempervirine.[2]

(6) (a) A. Kandiah, *J. Chem. Soc.*, **922** (1931). (b) W. Hückel and H. Friedrich, *Ann.*, **451**, 132 (1926).

(7) We wish to thank Dr. Janot for his kindness in making this sample available.

(8) This stereochemistry is further confirmed by the synthesis of *dl*-yohimbane by Van Tamelen and Shamma who independently carried out a similar series of transformations starting with *trans*-β-hydrindanone (see accompanying communication).

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RECEIVED JANUARY 15, 1954

A NEW METHOD FOR IDENTIFYING C-TERMINAL RESIDUES IN PEPTIDES

Sir:

Although there are several satisfactory methods for the identification of N-terminal residues in peptides,[1,2] there are few methods for the identification of C-terminal residues. We have recently investigated the thiohydantoin method discovered

(1) H. G. Khorana, *Quart. Rev.*, **6**, 340 (1952).

(2) P. Desnuelle, "Advances in Enzymology," Vol. 14, Interscience Publishers, Inc., New York, N. Y., 1953.