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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RECEIVED JANUARY 4, 1954

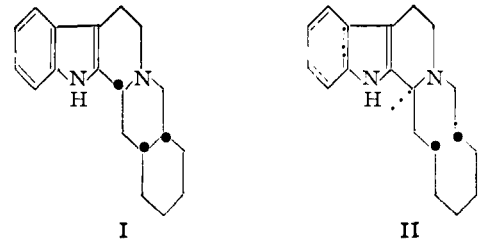
THE STEREOSPECIFIC SYNTHESIS OF *dl*-ALLOYOHIMBANE AND *dl*-3-EPIALLOYOHIMBANE

Sir:

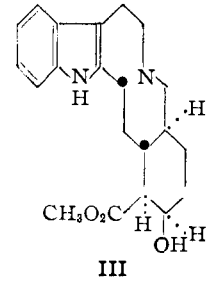
Three of the four possible steric arrangements of the ring system present in the yohimbe alkaloids have so far been found in nature: they are the ring systems present in yohimbane,[1] ψ-yohimbone[2] and alloyohimbane.[3]

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.[3]

(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.

by Schlack and Kumpf[3] and shown its applicability to peptides and proteins in the identification of their C-terminal residues on a micro scale.[4] The work of other investigators[5,6] as well as our studies has shown that some amino acids, notably aspartic acid, glutamic acid, serine, arginine and proline are not revealed by the thiohydantoin method. In a search for an alternative or complementary method we have found that the reaction of amino acids discovered by Dakin and West[7] can be used to identify the C-terminal residues in peptides. If a peptide is heated with acetic anhydride and pyridine, and the reaction product is then hydrolyzed, the hydrolyzate does not contain the C-terminal amino acid. If such a hydrolyzate is spotted on paper and chromatographed, the C-terminal residue of the original peptide is found to be absent, as shown for several peptides in Fig. 1. In each case the comparison of the hydrolyzate of the untreated peptide with that of the reaction product permits an identification of the C-terminal amino acid.

$$-\mathrm{NHCHR'CONHCHRCOOH} \xrightarrow[C_5H_5N]{(CH_3CO)_2O} CO_2 + -\mathrm{NHCHR'CONHCHRCOCH_3}$$

$$\xrightarrow{H_2O} \mathrm{NH_2CHR'COOH} + \mathrm{NH_2CHRCOCH_3}$$

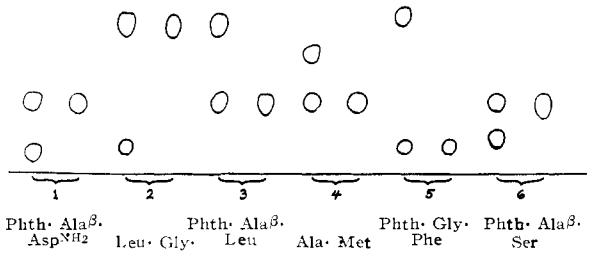

Fig. 1.—Drawing of paper chromatograms from six peptides: 1, phthaloyl-β-alanyl-DL-asparagine; 2, L-leucylglycine; 3, phthaloyl-β-alanyl-L-leucine; 4, DL-alanyl-DL-methionine; 5, phthaloylglycyl-DL-phenylalanine; 6, phthaloyl-β-alanyl-DL-serine. Of each pair of chromatograms the one on the left represents that obtained from a hydrolyzate of the peptide, while the one on the right represents that obtained from the hydrolyzate of the reaction product resulting from treatment of the peptide with acetic anhydride and pyridine.

It is noteworthy that phthaloyl-β-alanylserine gives a result that identifies serine as the C-terminal residue, while the thiohydantoin method failed to reveal a C-terminal residue in this peptide.[4]

The only previous example, in our knowledge, of an investigation of the reaction between a peptide and acetic anhydride in the presence of pyridine is that of Cleland and Niemann,[8] who showed that DL-alanylalanine evolved approximately the stoichiometric quantity of carbon dioxide.

(3) P. Schlack and W. Kumpf, *Z. physiol. Chem.*, **154**, 125 (1926).

(4) R. A. Turner and G. Schmerzler, *Biochem. Biophys. Acta*, in press.

(5) J. M. Swan, *Australian J. Sci. Research*, **A5**, 711, 721, 728 (1952).

(6) V. H. Baptist and H. B. Bull, THIS JOURNAL, **75**, 1727 (1953).

(7) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 91, 745, 757 (1928).

(8) G. H. Cleland and C. Niemann, THIS JOURNAL, **71**, 841 (1949).

In our general procedure 5 to 10 mg. of peptide, 0.75 ml. of acetic anhydride and 0.50 ml. of pyridine were heated in a sealed tube at 150° for two to three hours. The contents of the tube were rinsed out with water and evaporated to dryness. After solution in water and evaporation to dryness a second time, the residue was dissolved in 3 ml. of 6 *N* hydrochloric acid and heated at 110° overnight. The hydrolyzate was evaporated to dryness *in vacuo*, then alternately dissolved in water and evaporated three times more in order to diminish the quantity of hydrochloric acid. The final residue was dissolved in 0.2 ml. of water, of which 5 to 10 μ l. was chromatographed on paper.

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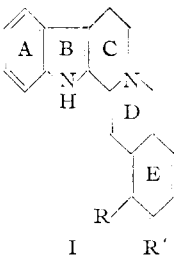
THE STEREOSPECIFIC SYNTHESIS OF *dl*-YOHIMBANE

Sir:

Yohimbane (I, R = R′ = H) has been obtained from the alkaloid *yohimbine* (I, R = COOCH$_3$; R′ = OH) by Oppenauer oxidation and



decarbomethoxylation to yohimbone[1] followed by Wolff–Kishner reduction,[2] a route which leaves unaltered the three remaining asymmetric centers in the derived molecule. The synthesis of *dl*-yohimbane described in this communication—apart from serving as a model for total synthesis—allows the unequivocal assignment of the relation between two of these centers, *viz.*, the carbon atoms common to rings D and E, as *trans*.[3,4]

dl-trans-Hydrindan-2-one,[5] on oxidation with perbenzoic acid, afforded the lactone of *dl-trans*-2-hydroxymethylhexahydrophenylacetic acid, m.p. 38–39° (Calcd.: C, 70.10; H, 9.15. Found: C, 70.35; H, 9.11); transformation of the lactone into ethyl *dl-trans*-2-bromomethylhexahydrophenylacetate, b.p. 129° at 1.4 mm. (Calcd.: Br, 30.3. Found: Br, 29.6) was accomplished by the action of alcoholic hydrogen bromide at room

(1) B. Witkop, *Ann.*, **554**, 105 (1943).

(2) J. Jost, *Helv. Chim. Acta*, **32**, 1301 (1949).

(3) B. Witkop (THIS JOURNAL, **71**, 2559 (1949)), basing his view on the results of certain drastic degradation experiments, has expressed a preference for the *trans* juncture of the D and E rings in yohimbine.

(4) A like conclusion has been reached by G. Stork and R. Hill (*ibid.*, **76**, 949 (1954)), who synthesized *dl*-alloyohimbane by a route similar to that outlined herein.

(5) R. S. Thakur, *J. Chem. Soc.*, 2147 (1932).