intestinal mucosa,[3] and that it may be an intermediate in the biosynthesis of cholesterol. Evidence for the existence of demethylated intermediates in the conversion of lanosterol to cholesterol have been reported.[15]

Acknowledgment.—We are indebted to Dr. Hans Noll for infrared measurements, and to Drs. Klaus Hofmann and Arvid Ek for helpful suggestions.

(15) F. Gautschi and K. Bloch, THIS JOURNAL, **79**, 684 (1957).

BIOCHEMISTRY DEPARTMENT
UNIVERSITY OF PITTSBURGH WILLIAM W. WELLS
SCHOOL OF MEDICINE DEWEY H. NEIDERHISER
PITTSBURGH, PENNSYLVANIA

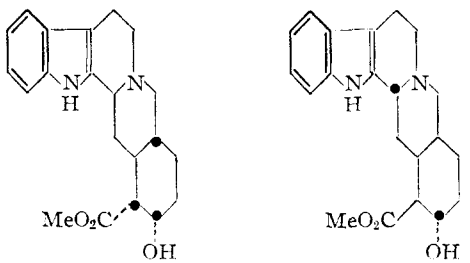
RECEIVED NOVEMBER 15, 1957

THE STEREOCHEMICAL INTERRELATIONSHIP OF THE YOHIMBINE-TYPE ALKALOIDS[1]

Sir:

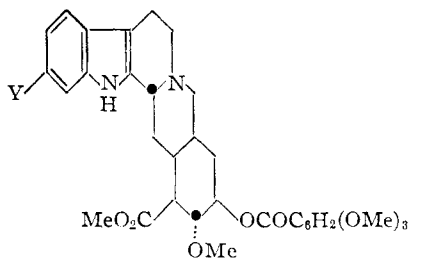
We wish to report the completion of the first phase of our continuing studies of the stereointerrelationship of indole alkaloids containing the ring system of yohimbine, ajmalicine and corynantheine, *i.e.*, the steric relationship of all yohimbine-type alkaloids.

Corynanthine, β-yohimbine and pseudoyohimbine have all been related to yohimbine,[2] whose absolute configuration (I) was ascertained by molecular rotation difference[3] and optical rotatory dispersion[4] studies. Similarly, alloyohimbine, rauwolscine, deserpidine, raunescine and isoraunescine have been interrelated with 3-epi-α-yohimbine[2,5,6]



I

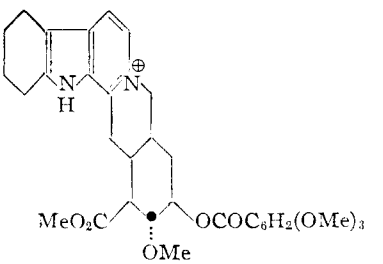
II



III

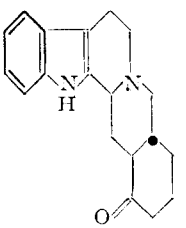
(II). Finally, rescinnamine and pseudoreserpine have been correlated with reserpine[2,6,7] (III, Y = OMe). However, no data chemically relating the three groups of compounds have been reported yet.

Palladium–maleic acid dehydrogenation[8] of reserpine (III, Y = OMe) yielded its tetradehydro derivative (47%) as perchlorate, m.p. 194–196° (Found for $C_{33}H_{37}O_{13}N_2Cl\cdot CH_3OH$: C, 55.11; H, 6.02; N, 3.80). Hydrogenation of the latter with platinum in acetic acid[9] gave the demethoxytetrahydrotetradehydro compound IV (61%), m.p. 188–189°, λ_{max} 250 mμ (log ε 4.16), 270 mμ (log ε 4.10) and 335 mμ (log ε 3.61), $[\alpha]_D$ −40° (chloroform) (Found for $C_{32}H_{35}O_{12}N_2Cl$: C, 55.94; H, 5.25; N, 4.25). The identical product, checked by m.p., mixed m.p., infrared and ultraviolet spectra, and specific rotation, was obtained by the catalytic hydrogenation of the tetradehydro derivative of deserpidine (III, Y = H).[8b] These results constitute the first chemical correlation of compounds of group II and III, and represent a potentially useful method of interrelating the many ring A oxygenated ajmalicine-type alkaloids with alstonine and serpentine.[2]

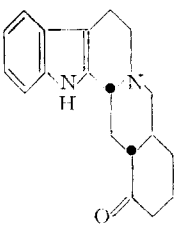


IV

When apoyohimbic acid hydrochloride, readily derivable from yohimbine[2] (I), was exposed to a Schmidt reaction, 16-yohimbone (V), m.p. 256° (dec.), $[\alpha]_D$ −86.1° (pyridine) (Found for $C_{19}H_{22}ON_2$: C, 77.7; H, 7.28; N, 9.3) was obtained in 17% yield.[10] The same reaction, carried out on the *apo* derivative of 3-epi-α-yohimbine[5a] (II), led to a ketone (17%) whose m.p. 254–256°, $[\alpha]_D$ +85.0° (pyridine), and infrared spectrum, identical with that of V, proved it to be the enantiomer of 16-yo-



V



VI

(1) This work was supported in part by a research grant from Ciba Pharmaceutical Products, Inc., and from the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare (M1301).

(2) For a review of the stereochemistry of indole alkaloids *cf.* J. E. Saxton, *Quart. Revs.*, **10**, 108 (1956).

(3) W. Klyne, *Chemistry and Industry*, 1032 (1953).

(4) C. Djerassi, R. Riniker and B. Riniker, THIS JOURNAL, **78**, 6362 (1956).

(5) (a) C. F. Huebner and E. Schlittler, *ibid.*, **79**, 250 (1957); (b) E. E. van Tamelen and C. W. Taylor, *ibid.*, **79**, 5256 (1957).

(6) For a discussion of the absolute configuration of these alkaloids based on molecular rotation difference data *cf.* E. Schlittler, The Chemistry of Rauwolfia Alkaloids, in "Rauwolfia," Little, Brown and Company, Boston, 1957.

(7) M. W. Klohs, F. Keller, R. E. Williams and G. W. Kusserow, THIS JOURNAL, **79**, 3763 (1957).

(8) (a) E. Wenkert and L. H. Liu, *Experientia*, **11**, 362 (1955); (b) E. Wenkert and D. K. Roychaudhuri, in press.

(9) *Cf.* H. Schwarz and E. Schlittler, *Helv. Chim. Acta*, **34**, 629 (1951).

(10) Since the completion of this work the synthesis of 16-keto-yohimbane has been reported also by R. K. Hill and K. Muench, *J. Org. Chem.*, **22**, 1276 (1957).

himbone (VI).[11] The correlation of yohimbine-type alkaloids with D/E *trans* fusion with those of D/E *cis* juncture thus is accomplished.

The above data illustrate unambiguously for the first time that the only center of asymmetry common to all yohimbine-type alkaloids is C-15.[12,13]

(11) The Schmidt reaction of tetrahydroserpentic acid and tetrahydroalstonic acid does not lead to ketones directly, but instead produces stable isocyanates. These results and further data will be discussed at a later time.

(12) This fact was pointed out by A. K. Bose, B. G. Chatterjee and R. S. Iyer [*Ind. J. Pharm.*, **18**, 185 (1956)] in connection with their discussion of the absolute configuration of yohimbine alkaloids, based on molecular rotation difference data.

(13) The authors are most grateful to Drs. Huebner, Lucas, MacPhillamy and Schlittler for a generous supply of the compounds needed for this study and to the Institute of Atomic Research, Ames, Iowa, for the use of a Baird infrared spectrophotometer.

DEPARTMENT OF CHEMISTRY ERNEST WENKERT
IOWA STATE COLLEGE ERNEST W. ROBB
AMES, IOWA N. V. BRINGI

RECEIVED NOVEMBER 6, 1957

OBSERVATIONS OF NEW PHENOMENA IN THE FLUORESCENCE SPECTRUM OF A DIPHOSPHOPYRIDINE NUCLEOTIDE-LINKED DEHYDROGENASE

Sir:

Boyer and Theorell[1] have reported that the wave length of the maximum intensity of the fluorescence spectrum of reduced diphosphopyridine nucleotide (DPNH) shifts from 450 mμ to about 415 mμ when DPNH combines with liver alcohol dehydrogenase. Inspection of the figure presented by these authors indicates that the intensity of fluorescence of the alcohol dehydrogenase–DPNH complex is approximately 1.8 times that of DPNH alone.

We have made similar measurements with crystalline heart muscle lactic dehydrogenase using the Aminco–Bowman recording spectrophotofluorimeter.[2] The fluorescent spectra shown in Fig. 1 were measured in 0.05 *M* phosphate buffer, *p*H 6.88, at room temperature using an activating wave length of 340 mμ. The curve labelled LDH was obtained with 8.4×10^{-7} *M* lactic dehydrogenase. The molecular weight of the enzyme was taken as 135,000.[3] The curve for DPNH was recorded at a DPNH concentration of 2.5×10^{-6} *M*. When DPNH and LDH were each present at the concentration used for the measurement of their separate spectra, the curve DPNH–LDH was obtained. The wave length of maximum fluorescent emission occurs at 430 mμ as compared to 455 mμ for DPNH. Preliminary experiments indicate that the maximum shift in fluorescence occurs when the ratio of DPNH concentration to LDH concentration is approximately four.

The addition of pyruvate to the DPNH–LDH system to a final concentration of 1.2×10^{-4} *M* results in a rapid change to the spectrum of LDH alone. Surprisingly, the addition of oxamate to the same concentration results in a similar change. Since oxamate has been shown to be a powerful inhibitor for the enzymatic reaction[4] and has been

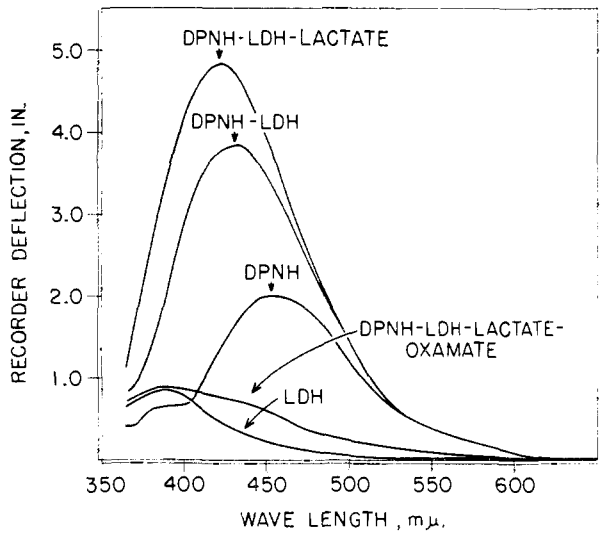


Fig. 1.

shown to be competitive with respect to pyruvate and non-competitive with respect to DPNH, it is inferred that this spectral change results from the formation of an LDH–DPNH–oxamate complex. The concentration of oxamate required to reduce the intensity of the LDH–DPNH fluorescent spectrum to half its initial value is approximately the value of K_I when oxamate is used as an inhibitor for the LDH-catalyzed reaction between DPNH and pyruvate, *i.e.*, approximately 10^{-5} *M*.

The curve labelled DPNH–LDH–lactate was obtained when Na-L-lactate, at a final concentration of 4.3×10^{-2} *M*, was added to LDH and DPNH present in the concentrations used for the other curves. The wave length of maximum emission is shifted to 420 mμ. The high concentration of lactate required to produce this effect, which presumably arises from an LDH–DPNH–lactate complex, is consistent with the observation that lactate has essentially no effect as an inhibitor of the LDH-catalyzed reaction between DPNH and pyruvate. The addition of oxamate to a concentration of 2×10^{-4} *M* results in the curve labelled DPNH–LDH–LACTATE–OXAMATE.

Results previously published from this Laboratory[5,6] indicate that a ternary LDH–DPNH–pyruvate complex is the reactive intermediate in the reaction catalyzed by LDH. Kinetic results also indicate that oxamate acts as an inhibitor by forming an unreactive LDH–DPNH–oxamate complex. Since the addition of diphosphopyridine nucleotide (DPN), which does not itself fluoresce, to LDH causes no change in the fluorescent spectrum of LDH, the present results suggest that in the LDH–DPNH–oxamate complex the bonding electrons of DPNH are constrained in a configuration resembling the aromatic ring of DPN. Moreover, since no net reaction occurs, it is possible that the LDH–DPNH–oxamate complex is an abortive complex similar in structure to the activated complex formed between LDH, DPNH and pyruvate.

(1) P. D. Boyer and H. Theorell, *Acta Chem. Scand.*, **10**, 447 (1956).

(2) R. L. Bowman, P. A. Caulfield and S. Udenfriend, *Science*, **122**, 32 (1955).

(3) J. B. Neilands, *J. Biol. Chem.*, **208**, 225 (1954).

(4) M. T. Hakala, A. J. Glaid and G. W. Schwert, *Federation Proc.*, **12**, 213 (1953).

(5) M. T. Hakala, A. J. Glaid and G. W. Schwert, *J. Biol. Chem.*, **221**, 191 (1956).

(6) Y. Takenaka and G. W. Schwert, *ibid.*, **223**, 157 (1956).