formed during the reaction. The reaction mixture was frozen in carbon dioxide and the hydrogenation apparatus was evacuated and flushed several times with deuterium gas before it was finally evacuated. The reaction took place at room temperature. The tritium gas (2 Curie, T_2/H_2 ratio = 78%, volume 1.09 ml., Harwell) was allowed to react first for 1 hour, then small volumes of deuterium gas were introduced at intervals into the reaction bottle so that the hydrogenation was completed.

The catalyst was filtered off and washed three times with 0.1 ml. of dioxane. The filtrate was diluted with 15 ml. of distilled water. During vigorous stirring the *p*H of 7.2 was adjusted to 4.6 with 0.2 ml. of 0.1 *N* sulfuric acid. The stirring was continued for four hours. The solution was kept at +4° overnight and filtered the following day. The filtrate was washed twice with 0.5 ml. of distilled water.

To remove freely exchangeable tritium the product was dissolved in 15 ml. of distilled water and acidified to *p*H 2 with sulfuric acid. After stirring for ten minutes the base was recrystallized by adjusting the *p*H to 4.6 with dilute sodium hydroxide.

The yield was 28 mg. of tetracycline base. The product was analyzed by ultraviolet spectrophotometry.[4] The remaining chlorotetracycline was determined fluorimetrically.[5]

Result.—Tetracycline 98.14%, chlorotetracycline 1.86%. Radioactivity measurement of the tetracycline as an infinitely thin layer was performed in a Tracerlab SC 16 windowless gas flow counter. Comparison with a tritium standard measured under identical conditions resulted in a specific activity of 0.25 mC./mg.

We are indebted to Eng. H. Thelin and Civ. Eng. L. Nathorst Westfelt, AB Astra, Södertälje, for valuable help.

The investigation has been financially supported by a grant from the Knut and Alice Wallenberg Foundation.

(4) "United States Pharmacopoeia," Vol. XV, p. 725.
(5) B. Örtenblad, personal com.

STOCKHOLM, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD UNIVERSITY AND THE CHANDLER LABORATORY OF COLUMBIA UNIVERSITY]

The Stereospecific Synthesis of *dl*-Alloyohimbane and *dl*-3-Epialloyohimbane[1]

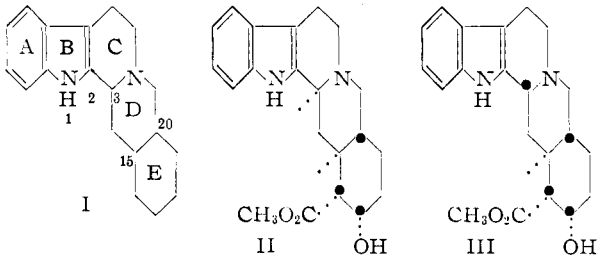
BY GILBERT STORK AND RICHARD K. HILL

RECEIVED JULY 30, 1956

A general method for the stereospecific synthesis of the alloyohimbane skeleton is described. This has led to the first synthesis of the pentacyclic ring system present in reserpine. The stereochemistry of the two alloyohimbanes is established.

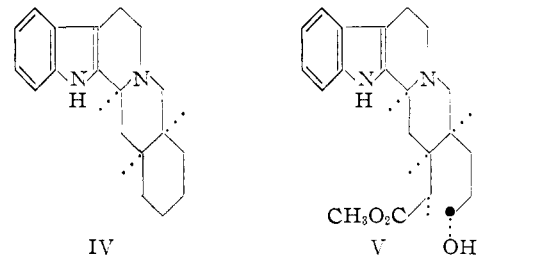
This paper reports the establishment of the stereochemistry of alloyohimbane (IV) and 3-epialloyohimbane (VI) and the development of a stereospecific synthetic route to the pentacyclic nucleus present in reserpine and related alkaloids.

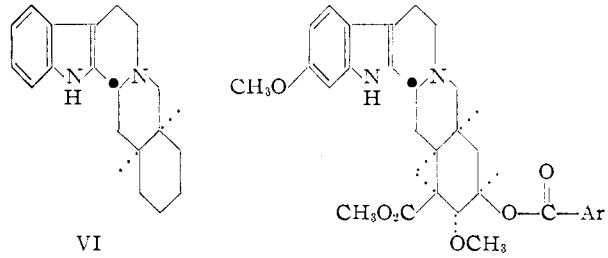
At the time this work was reported[1] only three of the four possible stereochemical arrangements of the pentacyclic system (I) had been encountered in nature. In two of these the junction between rings D and E is *trans* and the two possible configurations at C_3 can be illustrated by yohimbine (II) and ψ-yohimbine (III).[2]



The other two possible arrangements of the skeleton shown in I have a *cis* fused D/E system and again two possible configurations at C_3. The alloyohimbane system (IV) is found, for instance, in alloyohimbine and α-yohimbine (V).

Remarkably, the system of the 3-epimer of IV, which we have termed 3-epialloyohimbane (VI), was encountered in nature only after we had reported its synthesis[1]: its presence was demonstrated by Bader, *et al.*,[3] in "alkaloid 3078" isolated from *Rauwolfia serpentina* and eventually identified as 3-epi-α-yohimbine. Later, deserpidine and reserpine (VII) were identified as members of the new series by MacPhillamy, Huebner, Schlittler, St. André and Ulshafer.[4]



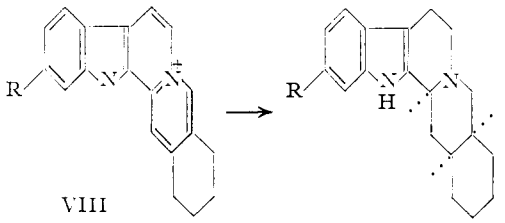


VII, Ar = 3,4,5-trimethoxyphenyl

We now turn to the problem of the stereospecific synthesis of IV and VI and note that the only

(1) G. Stork and R. K. Hill, THIS JOURNAL, **76**, 949 (1954).
(2) For a recent review see J. E. Saxton, *Quart. Rev.*, **10**, 108 (1956).
(3) F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, THIS JOURNAL, **77**, 3547 (1955).
(4) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *ibid.*, **77**, 4335 (1955).

method previously available for the synthesis of the D/E *cis* pentacyclic system is that due to Le-Hir, Goutarel and Janot[5] who succeeded in obtaining alloyohimbane by the catalytic hydrogenation of sempervirine (VIII, R = H).



VIII

The same method has been used by Huebner, St. André, Schlittler and Uffer[6] to synthesize 11-methoxyalloyohimbane, a degradation product of reserpine, from VIII (R = methoxy).

This synthetic approach seemed to us to suffer from two drawbacks: It is not readily applicable to substances substituted in ring E and it is not useful as a means of establishing the stereochemistry of alloyohimbane because of the uncertainties attending the course of the hydrogenation of VIII.[1,7] Thus, although Le Hir, Goutarel and Janot[5] correctly assigned stereochemistry IV to the hydrogenation product of VIII, this assignment was later changed to VI by Janot, Goutarel, Le-Hir, Tsatsas and Prelog.[7]

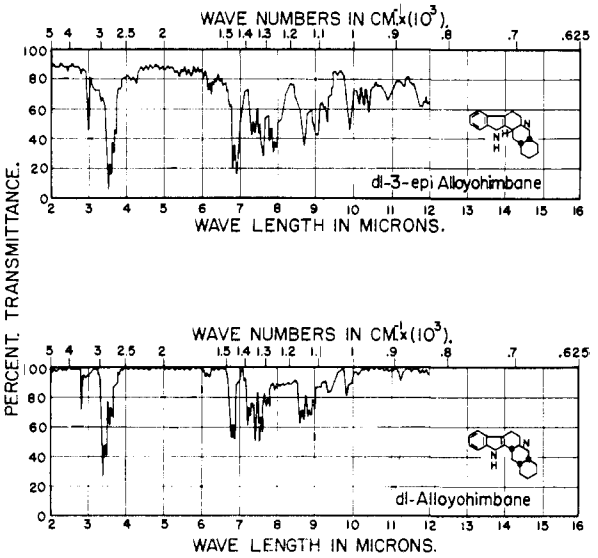
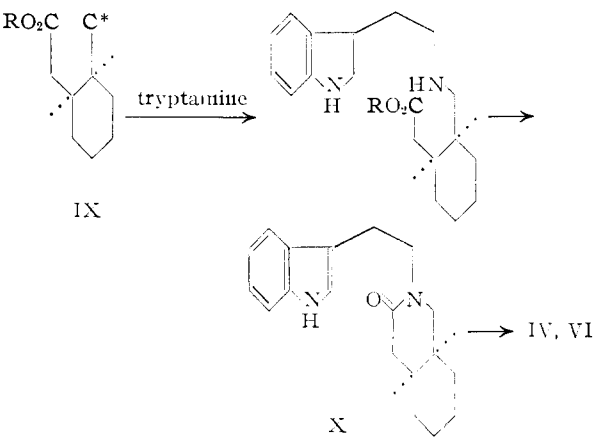
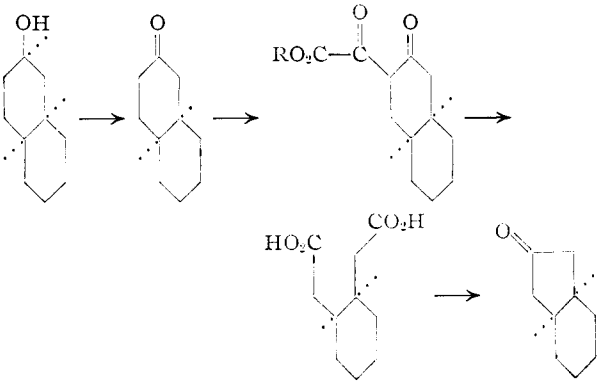


Fig. 1.—Infrared spectra in Chloroform solution.

The synthetic scheme which we developed involves the elaboration of a system such as IX in which the starred carbon atom is capable of forming a carbon–nitrogen bond with tryptamine. Subsequent or simultaneous lactam formation would lead to X which we hoped would undergo cyclization and reduction to the desired substances IV and VI.[8]



The starting material for this synthesis was *cis*-β-hydrindanone. This has been prepared previously *via* the steps β-naphthol → *cis*-β-decalol (m.p. 105°)[9] → *cis*-β-decalone[10] → *cis*-cyclohexane-1,2-diacetic acid[11] → *cis*-β-hydrindanone.[12] The published procedure was followed, but the direct oxidation of *cis*-β-decalone with nitric acid is unsatisfactory, as it produces mainly the undesired 2-carboxycyclohexanepropionic acid. This difficulty was overcome by condensation of the decalone with ethyl oxalate and ozonolysis of the resulting glyoxylate



It is interesting to note that oxidation to a carboxypropionic acid involves the favored enol of *cis*-β-decalone while condensation with ethyl oxalate proceeds *via* the *less stable* enolate ion

This phenomenon is noteworthy but not surprising: The determining factor is clearly that the intermediate state in reactions such as Claisen condensations (or aldol formation, etc.) is extremely bulky, and the equilibrium is consequently affected markedly by steric factors—in the present

(5) A. LeHir, M.-M. Janot and R. Goutarel, *Bull. soc. chim.*, 1091 (1952).

(6) C. F. Huebner, A. F. St. André, E. Schlittler and A. Uffer, THIS JOURNAL, **77**, 5725 (1955).

(7) M.-M. Janot, R. Goutarel, A. LeHir, G. Tsatsas and V. Prelog, *Helv. Chim. Acta*, **38**, 1073 (1955).

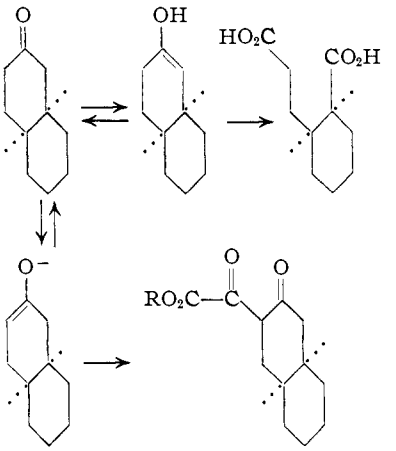
(8) Previous attempts to utilize related schemes have been rather unsuccessful: *cf.* J. Jost, *ibid.*, **32**, 1297 (1949); G. A. Swan, *J. Chem. Soc.*, 1720 (1949). The present synthetic method which we recorded earlier[1] and which is described in detail here is a new synthesis of the pentacyclic system of the yohimbane alkaloids. Its usefulness and generality is well illustrated by its subsequent use in the total synthesis of reserpine (R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey and R. W. Kierstead, THIS JOURNAL, **78**, 2023 (1956)).

(9) W. Hückel, *Ann.*, **451**, 109 (1926).

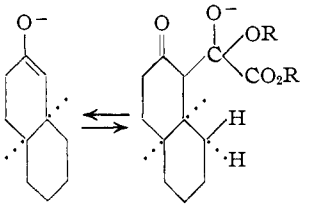
(10) K. A. N. Rao, *J. Chem. Soc.*, 1954 (1929).

(11) A. Kandiah, *ibid.*, 922 (1931).

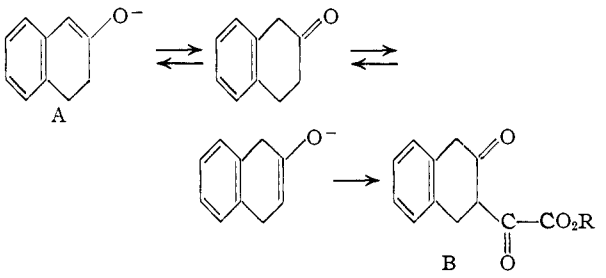
(12) W. Hückel and H. Friedrich, *Ann.*, **451**, 132 (1926).



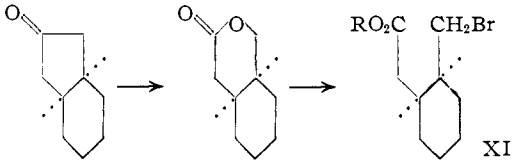
case by interference with the hydrogens in the *peri* position.



Perhaps the most striking example of this steric control of Claisen type reactions is found in the condensation of β-tetralone with ethyl oxalate[13]: in spite of the much lower energy of anion A, condensation leads to B

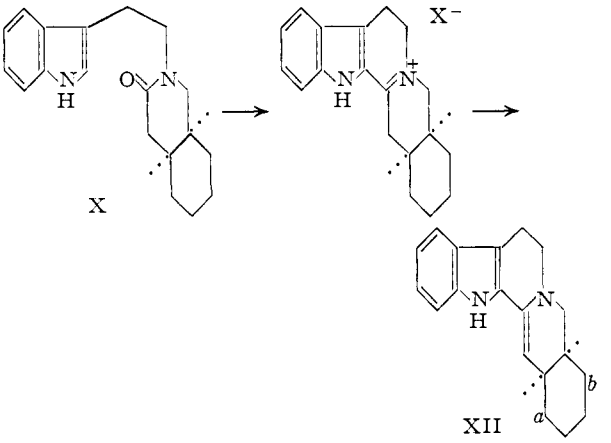


Elaboration of *cis*-β-hydrindanone toward a substance of type IX was achieved by transforming the cyclopentanone ring into a six-membered lactone by treatment with perbenzoic acid. Refluxing the lactone with ethanol saturated with anhydrous hydrogen bromide produced the desired *cis*-2-bromomethylcyclohexaneacetic acid (XI = IX) as its ester.



It was now possible to form the lactam X: this was accomplished by refluxing the bromoester XI with tryptamine in dimethylformamide solution, in the presence of sodium iodide. The *cis*-2-[2-(3-indolyl)-ethyl]-octahydro-3(2H) isoquinolone (X) was purified by chromatography and melted at 171–172°. It had the characteristic infrared absorption band of a six-membered N-substituted lactam at 6.15μ.

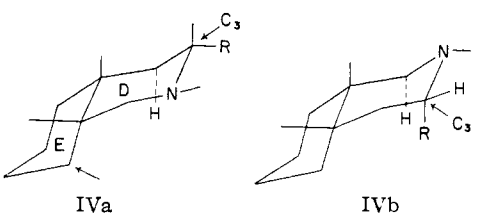
Closure of the C ring was now effected by refluxing with phosphorus oxychloride in an atmosphere of nitrogen, and the free base XII obtained from the quaternary salt was used in reduction experiments without purification, as it was found to be rather unstable.



At this stage we have to consider the introduction of the third asymmetric center. Catalytic hydrogenation with platinum oxide in ethanol led to a rapid uptake of hydrogen and a single substance was isolated which had m.p. 143.5–144°. This proved to be *dl*-alloyohimbane (IV) by comparison of infrared spectra and melting points with an authentic sample kindly supplied by Dr. Janot.[5]

The stereochemistry of this reduction product can only be that shown in IV (hydrogen at 3 *cis* to those at 15 and 20). The classic work of Linstead[14] has demonstrated that the stereochemistry of hydrogenation is such that hydrogen is added from the catalyst to the side of the molecule which is adsorbed on it. In many cases, it is not easy to decide on which side a given molecule will be adsorbed on a catalyst surface. In the present case, there are two possible conformations of XII which must be considered: the *cis* junction of rings D and E permits having either the branch marked *a* or that marked *b* (see XII) axial to the D ring. Consequently, approach of hydrogen is blocked from the side of *a* or *b* and the reduction product must have all *cis* stereochemistry. The stereochemistry of alloyohimbane is thus definitely that shown in IV.

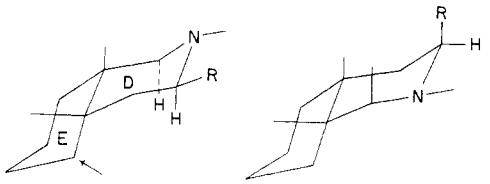
We may note at this point that the stereochemistry of alloyohimbane can be represented by IVa



(13) M. D. Soffer, R. A. Stewart and G. L. Smith, THIS JOURNAL, **74**, 1556 (1952).

(14) R. P. Linstead, *et al.*, *ibid.*, **64**, 1985 (1942).

or IVb while the 3-epimer can have conformation VIa or VIb. The stable conformation of either

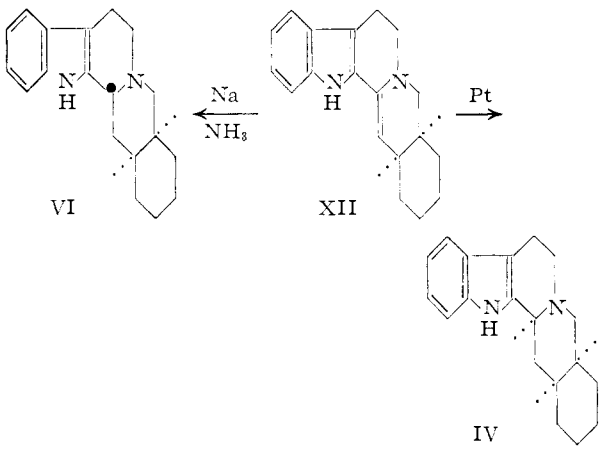


VIa IVb

C-3 epimer is certainly that in which the bulky indole complex at C_3 (designated R) is equatorial, and consequently the stable conformation in the alloyohimbane series is IVa and, similarly, VIa is the lower energy arrangement in the 3-epialloyohimbane series.

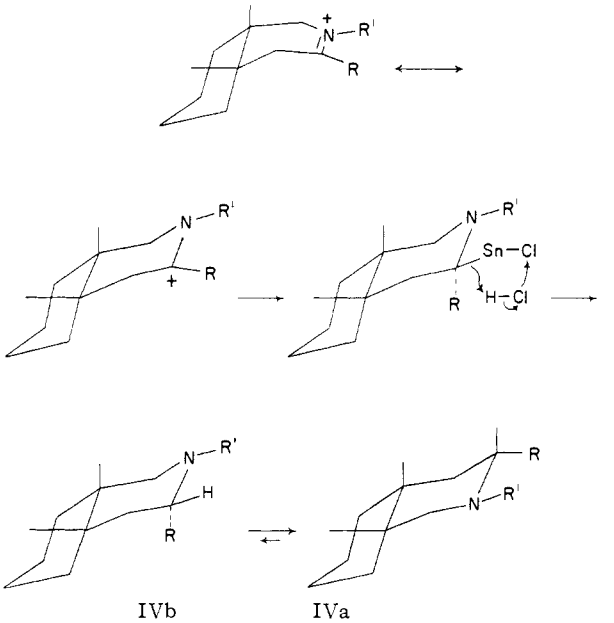
It is now of interest that the only difference between IVa and VIa is that of the ring E methylene group which is axial to ring D (marked by an arrow) interferes with two axial hydrogens in VIa and with one axial hydrogen and a nitrogen electron pair in IVa. The more stable member of the pair should therefore be VIa.[15] In other words, 3-epialloyohimbane should be more stable than alloyohimbane.

In keeping with this conclusion, it was found possible to reduce the unsaturated amine **XII** with sodium and liquid ammonia, a reduction process which normally leads to the more stable of two possible epimers[16]: The main product of the reaction was a substance, m.p. 185–186°, isomeric with alloyohimbane, evidently *dl*-3-epialloyohimbane.



VI XII IV

Wenkert[17] has shown recently that equilibration at C_3 can be brought about by acid treatment of alloyohimbane and that the equilibrium reached under these conditions is also in favor of 3-epialloyohimbane. In that connection, a particularly interesting result was obtained in the reduction of XII with tin and hydrochloric acid: The less stable isomer, alloyohimbane (IV), was formed. The reaction must therefore be kinetically controlled, and we would like to suggest that the determining factor is hindrance to the approach of the metal by axial groups in the meta position to the electron-deficient carbon. In other words the metal enters equatorially and hydrolysis of the C-metal bond then takes place by an SNi mechanism, without inversion: This results in the formation of the less stable isomer (equatorial hydrogen, axial alkyl). The formation of alloyohimbane is illustrated as



IVb IVa

Reserpine is known to be a derivative of 3-epialloyohimbane.[4] The interesting fact has emerged that reserpine, although a derivative of 3-epialloyohimbane, is unstable with respect to its 3-epimer, isoreserpine, which belongs to the alloyohimbane series. The position of the 3-epialloyohimbane–alloyohimbane equilibrium is thus strikingly, and understandably, dependent on the stereochemical arrangement of the substituents in ring E.

Experimental

The tryptamine used in these experiments was made from the methosulfate of gramine, essentially by the method of Thesing and Schulde[18] except that the reduction of indole acetonitrile was carried out at 50° using one-quarter as much Raney nickel as compound. The yield of tryptamine was 92% and pure tryptamine, m.p. 115–116°, was obtained by sublimation.

cis-**2-Decalol.**—This was prepared by hydrogenation of one pound of technical β-naphthol in the presence of eight teaspoons of Raney nickel.[19] Hydrogenation was carried out at an initial pressure of 2300 lb. and a temperature of 140°. At the completion of the reduction, the filtered mixture was allowed to stand and deposited about 110 g. of *cis*-2-decalol, m.p. 100–102°. After one recrystallization from acetone the long white needles melted at 104–105° (reported[9] m.p. 105°).

cis-**2-Decalone.**—The decalol was oxidized by the method of Rao[10] to give *cis*-2-decalone, b.p. 77–79° at 2 mm., in 90% yield.

cis-**1,2-Cyclohexanediacetic Acid.**—Sodium methoxide was prepared from 35.2 g. of sodium and 350 ml. of methanol, followed by removal of the methanol at reduced pressure and drying at 180° at 1 mm. for 2 hr. The sodium

(15) *Cf.* D. H. R. Barton and R. C. Cookson, *Quart. Rev.*, **10**, 44 (1956).

(16) D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 3045 1954).

(17) E. Wenkert and L. H. Lui, *Experientia*, **11**, 302 (1955).

(18) J. Thesing and F. Schulde, *Ber.*, **85**, 324 (1952).

(19) *Org. Syntheses*, **21**, 15 (1941).

methoxide was suspended in 300 ml. of dry benzene, and a solution of 265 g. of ethyl oxalate in 300 ml. of benzene was added, followed by a solution of 233 g. of *cis*-2-decalone in 300 ml. of benzene. The flask was cooled in running water and shaken for 30 minutes while the solid dissolved and the mixture turned dark red. After standing overnight at room temperature the mixture was poured in ice-water, the benzene layer was separated and washed four times with water. The combined aqueous extracts were washed with ether, acidified in the cold with 1:3 hydrochloric acid and extracted with ether. After drying over sodium sulfate, the ether was removed at reduced pressure, leaving a light red oil weighing 305 g. (78%) which gave an immediate purple color with 1% ferric chloride solution.

A solution of 2.0 g. of the glyoxylate in 25 ml. of ethyl acetate was ozonized at −75°. The ozonide solution was added from a dropping funnel to 100 ml. of boiling water, and boiling was continued until disappearance of the ethyl acetate. Upon cooling, yellow crystals separated; these were filtered and the filtrate was extracted with chloroform. The total acid obtained weighed 830 mg. A series of ozonization of 20-g. batches of the glyoxylate, using 141 g. in all, gave 46.5 g. of pure *cis*-cyclohexane-1,2-diacetic acid, m.p. 160–161° after recrystallization from water (reported[11] m.p. 160°).

***cis*-2-Hydrindanone.**—The diacid above was converted to the cyclic ketone by the method of Kandiah, using barium oxide for the pyrolysis. This yielded 81% *cis*-2-hydrindanone, b.p. 127–128° (45 mm.). The semicarbazone had m.p. 215.5–216° (lit.[12] m.p. 215–216°).

Lactone of *cis*-2-(Hydroxymethyl)-cyclohexaneacetic Acid.—A solution of 0.36 mole of perbenzoic acid in 1250 ml. of chloroform was allowed to stand in the dark at room temperature for 10 days with 44.4 g. (0.32 mole) of *cis*-2-hydrindanone. The mixture was worked up by washing with 5% sodium bicarbonate solution, then with water and distillation after drying over sodium sulfate. The fraction boiling at 80–115° (5 mm.) was collected. Another distillation gave 39.7 g. (80%) of the pure lactone, b.p. 115–120° at 4 mm. The infrared spectrum showed the expected lactone bend at 5.79 μ.

Anal. Calcd. for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 70.37; H, 8.90.

Ethyl *cis*-2-(Bromomethyl)-cyclohexaneacetate.—A solution of 4.1 g. of the *cis*-lactone (0.026 mole) in 8.0 ml. of chloroform was saturated with gaseous hydrogen bromide in the cold. The increase in weight was 2.16 g., corresponding to 0.027 mole of hydrogen bromide. Absolute ethanol (4.7 ml.) and concentrated sulfuric acid (0.15 ml.) were added, and the mixture was refluxed for 12 hr. After washing with 5% sodium carbonate solution, then with water, the solution was dried over sodium sulfate and the solvent was evaporated. Distillation gave the *cis*-bromo ester, b.p. 100–106° at 1 mm. (4.0 g.).

Anal. Calcd. for $C_{11}H_{19}O_2Br$: C, 50.20; H, 7.28. Found: C, 50.45; H, 7.45.

***cis*-2-(Bromomethyl)-cyclohexaneacetic Acid.**—Attempts to hydrolyze the bromoester with 48% hydrobromic acid were unsuccessful, but the bromoacid could be obtained as follows: A chloroform solution of the *cis*-lactone (2.0 g.) was saturated with gaseous hydrogen bromide and allowed to stand in a stoppered vessel for 3 days at room temperature. The solvent was removed at reduced pressure, the residue was taken up in chloroform and was extracted with 50 cc. of 5% sodium bicarbonate solution. The alkaline solution was washed with ether and extracted with chloroform after acidification with 10% hydrochloric acid. Evaporation of the solvent left an oily residue which crystallized on standing in the cold. After washing with petroleum ether the material melted at 63–64°. Recrystallization from petroleum ether raised the melting point to 65.5–66°. An analytical sample was recrystallized from cyclohexane and dried *in vacuo*.

Anal. Calcd. for $C_9H_{15}O_2Br$: C, 45.97; H, 6.43. Found: C, 46.12; H, 6.64.

Reaction of Tryptamine with Bromoesters. (a) N-[2-(3-Indolyl)-ethyl]-pyrrolidone.—A solution of 0.97 g. of ethyl 4-bromobutyrate (10 mmoles) and 1.6 g. of tryptamine (10 mmoles) in 50 cc. of methyl Cellosolve was refluxed for 72 hr. Two-thirds of the solvent was removed by distillation; the residue was poured into water and extracted with chloroform. After washing with 2% hydrochloric acid and drying, the extracts yielded 1.27 g. (82%) of the lactam, m.p. 133–135°. The use of benzene or ethanol as solvent reduced the yield. Recrystallization from benzene and from methanol gave colorless rhombs, m.p. 136–137°. The infrared spectrum had the characteristic five-membered lactam band at 5.95 μ.

Anal. Calcd. for $C_{14}H_{16}ON_2$: C, 73.65; H, 7.07. Found: C, 73.82; H, 7.27.

(b) *cis*-2-[2-(3-Indolyl)-ethyl]-octahydro-3(2H)isoquinolone.—A solution of 2.5 g. (15.6 mmoles) of tryptamine, 2.0 g. (7.6 mmoles) of sodium iodide in 50 cc. of dimethylformamide was refluxed for 24 hr. The dark reaction mixture was poured into 250 cc. of ice-water containing 50 cc. of 1:4 hydrochloric acid. The mixture was extracted with 200 cc. of chloroform in three portions. The extracts were washed with water, dried over sodium sulfate and evaporated to dryness. The infrared spectrum showed the presence of considerable quantities of unreacted ester (5.78 μ) and some uncyclized amide (6.0 μ) in addition to the desired lactam (6.15 μ). Pure lactam was initially obtained from the mixture by chromatography on alumina in 40:1 ether–chloroform. The crystalline lactam appeared in early fractions and was thus obtained in about 20% yield, m.p. 170–171° (copper block). Recrystallization from methanol gave the analytical sample, m.p. 171–172°.

Anal. Calcd. for $C_{19}H_{24}ON_2$: C, 76.99; H, 8.16. Found: C, 76.87; H, 8.02.

In subsequent experiments the ether extract containing the crude neutral fraction was seeded with the crystalline lactam, thus avoiding the need for chromatography.

***cis*-2-[2-(3-Indolyl)-ethyl]-decahydroisoquinoline.**—A solution of 230 mg. of the *cis*-lactam in 50 cc. of dry tetrahydrofuran was added slowly to a stirred refluxing solution of 1.6 g. of lithium aluminum hydride in 100 cc. of tetrahydrofuran. Refluxing was continued for 45 hr., and the cooled solution was treated with 100 cc. of 10% sodium hydroxide solution. Removal of the tetrahydrofuran under reduced pressure left an alkaline solution which was extracted with chloroform. The extracts were washed with water, dried over sodium sulfate and the solvent was distilled off. The oily residue was chromatographed in ether over alumina. The product could not be crystallized and the largest fraction was converted to its methiodide which was obtained as a crystalline solid (200 mg.), m.p. 221–230°. Two recrystallizations from methanol gave white crystals melting at 234–236° (hot-stage).

Anal. Calcd. for $C_{20}H_{29}N_2I$: C, 56.60; H, 6.89. Found: C, 56.72; H, 7.01.

Before carrying out cyclization with phosphorus oxychloride, a model experiment was carried out with N-[2-(3-indolyl)-ethyl]-pyrrolidone to see whether closure could be effected by inverse reduction with lithium aluminum hydride which might lead to closure at the carbinolamine stage.[20] This was unsuccessful: To a refluxing and vigorously stirred solution of 1.0 g. of the pyrrolidone in 150 cc. of dry tetrahydrofuran was added dropwise over a period of 1.5 to 2 hr., a solution of 1.0 g. of lithium aluminum hydride in 100 cc. of tetrahydrofuran. After refluxing for 1 to 2 hr. longer, the mixture was cooled and decomposed with a saturated sodium sulfate solution, magnesium sulfate was added to coagulate the alumina and the solution was filtered. The filtrate was evaporated to a small volume, taken up in 100 cc. of 2% hydrochloric acid and washed with ether, then made alkaline with 10% sodium hydroxide solution and extracted with ether. After washing with water and drying, the ether was evaporated; the yield of crude product varied from 80 to 90%. In this way, N-[2-(3-indolyl)-ethyl]-pyrrolidine was obtained, m.p. 109–110°, after sublimation *in vacuo*. The Hopkins–Cole test on this reduced base gave a dark blue color. Under the same conditions, 2,3-dimethylindole gave a negative test.

Anal. Calcd. for $C_{14}H_{18}N_2$: C, 78.46; H, 8.47. Found: C, 78.69; H, 8.83.

***dl*-$\Delta^{3(14)}$-Dehydroalloyohimbane.**—A solution of 308 mg. of the *cis*-lactam in 8.0 cc. of redistilled phosphorus oxychloride was refluxed under nitrogen for 3 hr. The solution

(20) *Cf.* F. Galinovsky and R. Weiser, *Experientia*, **5**, 377 (1950).

quickly turned red, then lightened to yellow. Removal of the phosphorus oxychloride at reduced pressure gave a glass which was treated with 5% sodium hydroxide solution to liberate the base which was then extracted with chloroform. Even under nitrogen, the chloroform solution turned bright red during the extraction; but the color could be removed by shaking with a little charcoal. After drying over sodium sulfate the solvent was removed under nitrogen.

The unsaturated base was so sensitive to oxidation that it was reduced immediately, without purification.

dl-**Alloyohimbane.** (a) **By Platinum Reduction.**—The unsaturated base from 308 mg. of *cis*-lactam was taken up in 10 cc. of absolute ethanol and hydrogenated at atmospheric pressure with 15–20 mg. of platinum oxide. Within 15 minutes 19.2 cc. of hydrogen was absorbed. The mixture was filtered, the solvent was removed and the residue was chromatographed in ether over alumina.

The reduced base appeared in the first two fractions, weighing 120 mg., and melting at 147–149° (hot-stage) after recrystallization from methanol. An analytical sample was recrystallized three more times from methanol and dried 6 hr. at 100° *in vacuo*, m.p. 143.5–144° (capillary).

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63. Found: C, 81.44; H, 8.68.

(b) **By Reduction with Tin and Hydrochloric Acid.**—The unsaturated base from 385 mg. of the *cis*-lactam was dissolved in 15 cc. of ethanol and 10 cc. of concentrated hydrochloric acid and the mixture was refluxed gently. Over a period of 2 hr., 2.0 g. of mossy tin was added in small pieces. The solution slowly lightened from deep red to yellow. After refluxing for 2 more hr. water was added and the alcohol was removed under reduced pressure. Addition of 200 cc. of 10% sodium hydroxide solution and extraction with chloroform, followed by washing with water, drying over sodium carbonate and removal of the solvent left a residue which was chromatographed on alumina in 50/50 ether–petroleum ether. The *dl*-alloyohimbane crystallized from the second fraction, weighing 100 mg., and had m.p. 142–146°. After recrystallization from methanol it melted at 147–149° (hot-stage), alone or mixed with the base from the platinum reduction. Comparison of their infrared spectra confirmed their identity.

Comparison of both bases with racemic alloyohimbane from sempervirine was carried out, using a sample kindly furnished by Dr. Janot.[5] The reported melting point of 134° was raised after two recrystallizations from methanol to 142–146° (hot-stage). A mixture with our synthetic base, m.p. 147–149° (hot stage), had m.p. 144–148° (hot stage). The infrared spectra of the two substances were superimposable.

dl-**3-Epialloyohimbane.**—The unsaturated base from 300 mg. of the *cis*-lactam was dissolved in 250 cc. of liquid ammonia in a Dewar flask. One gram of freshly cut sodium and 3 cc. of anhydrous *t*-butyl alcohol were added in six portions over 1 hr., with a continuous stirring. After stirring for an additional hour, 0.3 g. of sodium was added and stirring was continued for another hour. Methanol was then added dropwise until the blue color disappeared and the ammonia was allowed to evaporate.

The residue was diluted with water and extracted with chloroform. After washing with water, treating with charcoal and drying over sodium carbonate, the solvent was removed, and the residue was chromatographed over 5 g. of alumina in 50/50 ether–petroleum ether. The second fraction gave 100 mg. of beautiful white needles which melted at 185–186° (hot-stage) after three recrystallizations from methanol.

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63. Found: C, 81.66; H, 8.65.

NEW YORK 27, N. Y.

COMMUNICATIONS TO THE EDITOR

ON THE ORIGIN OF C_{28} IN ERGOSTEROL

Sir:

There is considerable evidence that the biological syntheses of ergosterol and cholesterol follow the same general plan and branch from each other only at a relatively late stage. As has been shown in a study with an acetate-less mutant of neurospora, at least 26 of the 28 carbon atoms of ergosterol stem from acetic acid.[1] The distribution of isotope in biosynthetic ergosterol is only partially known but since C_{23} and C_{25}[2,3] and C_{11} and C_{12}[4,5] are derived from acetate-carboxyl in both cholesterol and ergosterol, it seems reasonably certain that the patterns are the same in both instances. On the other hand, the origin of C_{28}, the methyl substituent in the ergosterol side-chain, has hitherto remained obscure. C_{28} does not become labeled when 1-C^{14}-acetate is the precursor of ergosterol in yeast,[3] nor is the methyl carbon of acetate utilized, at least for C_{28} of the similarly side-chain substituted eburicoic acid.[6] We now report results showing that formate is an efficient carbon source for C_{28} of ergosterol. Yeast was suspended in a medium containing non-isotopic glucose plus C^{14} formate, and the ergosterol isolated from the cells after an incubation of 24 hours. Ozonization of the sterol yielded α,β-dimethylbutyraldehyde and this was further degraded by way of methyl isopropyl ketone to afford C_{28} as iodoform.[3] The specific activities listed in the table for ergosterol, the DNP derivative of the C_6-aldehyde, the semicarbazone of methyl isopropyl ketone and for iodoform clearly show that C^{14} from formate enters only the sterol side chain and that the C^{14} content of C_{28} accounts, within the limits of error, for all the radiocarbon incorporated into ergosterol. That formate is a carbon source specifically for C_{28} is further borne out by the finding that the non-saponifiable lipids of formate-incubated cells yielded on chromatography a single radioactive peak and this coincided with the ergosterol fraction. There was no evidence for the presence of radioactive squalene, zymosterol or lanosterol, all of which are

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