TABLE I

COFACTOR REQUIREMENTS FOR THE REDUCTION OF GLYCINE-2-C^{14} TO ACETATE-2-C^{14}

The complete system contained tris-(hydroxymethyl)-aminomethane (TRIS) buffer, (*p*H 8.7) 25 μmoles; $MgSO_4$, 3 μmoles; DPN, 0.2 μmole; pyridoxal phosphate, 0.006 μmole; DTP, 20 μmoles; 0.2 μC 2-C^{14}-glycine (*ca.* 30,000 cts./min.), 10 μmoles and 8 mg. protein. Reactants in 0.5 ml. final volumes were incubated anaerobically at 31° for two hours.

Omission	Acetate-2-C^{14} formed, cts./min.[3]
...	940
DPN	225
Mg^{++}	465

TABLE II

THE EFFECT OF ORTHOPHOSPHATE (PO_4), ARSENATE (AsO_4) AND ADENYLATE NUCLEOTIDES ON THE CONVERSION OF GLYCINE TO ACETATE

In addition to the reactants each sample contained TRIS buffer (*p*H 8.7), 20 μmoles; $MgCl_2$, 3 μmoles; DPN, 0.1 μmole; pyridoxal phosphate, 0.003 μmole; DTP, 9 μmoles; 0.2 μC glycine-2-C^{14}, 10 μmoles and 3.6 mg. protein (30 to 35% satd. $(NH_4)_2SO_4$ fraction), in a final volume of 0.5 ml. Incubations were carried out anaerobically at 31° for 90 min.

Experiment	Additions, μmoles	Acetate-2-C^{14} formed, μmoles[3]
1	None[a]	0.09
	PO_4 10, ADP 5, or AMP 5 or ATP 5	1.18 1.30 1.28
	PO_4 10, ADP 10	1.45
	PO_4 10, AMP 5, ATP 5	1.60
2	None[a]	0.25
	PO_4 10	0.56
	ADP 1	1.01
	PO_4 10, ADP 1	1.87
3	PO_4 5[a]	0.43
	AsO_4 5	1.29
	AsO_4 5, PO_4 5	1.42
	AsO_4 10	1.42
	AsO_4 10, AMP 5	1.40

[a] The enzyme preparation (not dialyzed) contained 0.88 μmole of orthophosphate per 3.6 mg. protein employed.

(Table III) shows that the reaction can be described by the equation

$$CH_2NH_2COOH + PO_4 + ADP + R(SH)_2 \longrightarrow CH_3COOH + NH_3 + ATP + RSS \quad (1)$$

The most significant result of these experiments is that for each mole of glycine converted to acetic acid and ammonia there is a concomitant esterification of one mole of orthophosphate which is incorporated into ATP.

Reduction of glycine in the presence of P^{32}-labelled orthophosphate and ADP (or AMP) results in a marked synthesis of P^{32}-labelled ATP. No labelled ATP is detected chromatographically[4] when glycine is omitted from the otherwise complete system or when amino acids such as citrulline, lysine or proline[5] are substituted. The failure to observe any phosphorylation associated with the reduction of proline to δ-aminovalerate by DTP,[1] a reaction also catalyzed by this enzyme fraction, suggests that the phosphorylation associated with glycine reduction is not solely the result of dithiol oxidation.

TABLE III

GLYCINE REDUCTION BALANCE EXPERIMENTS

Reaction mixture components as in Table II with AMP, 5 μmoles and K_2HPO_4, 3–10 μmoles. The enzyme preparation used to measure NH_3 formation had been precipitated with satd. Na_2SO_4 and redissolved in buffer to lower its $(NH_4)_2SO_4$ concentration. The amount of DTP oxidized was measured in incubation mixtures reduced to one-half the usual volume; all components were added in proportionally smaller amounts except for enzyme and glycine.

Glycine[6] dec., μmoles	$(SH)^7$ oxid., μ equiv.	PO_4[8] uptake, μmoles	P_{i^9} min. formed, μmoles	Acetate[3,9] formed, μmoles	NH_3[10] formed, μmoles
0.85	[b]	0.71	0.89	1.03[a]	0.77
1.01	[b]	1.07	0.98	1.01	1.23
[b]	2.52	1.25	0.89	1.21	[b]
[b]	2.30	0.98	0.98	1.0	[b]

[a] Glycine, 5 μmoles, present.
[b] Not measured.

The balance data presented, taken together with the fact that there is a compound formed that can be arsenolyzed, make it evident that the reductive deamination of glycine must involve the formation of a high energy phosphorylated intermediate. An unstable glycine derivative that is accumulated by another enzyme fraction derived from *C. sticklandii* may furnish a clue as to the nature of this intermediate.

(3) Residual C^{14}-glycine was removed by treatment with Dowex-50-H^+ resin at *p*H 1–2 and aliquots of the supernatant solutions assayed for C^{14} after neutralization. Identity of the radioactive product was established by steam distillations and Duclaux distillations.

(4) L. V. Eggleston and R. Hems, *Biochem. J.*, **52**, 156 (1952).

(5) T. C. Stadtman, *J. Bact.*, **67**, 314 (1954).

(6) E. C. Cocking and E. W. Yemm, *Biochem. J.*, **58**, xii (1954).

(7) P. D. Boyer, THIS JOURNAL, **76**, 4331 (1954).

(8) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(9) Acetate was also estimated as acethydroxamate after incubation with the acetyl kinase system of I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954).

(10) Ammonia was measured by direct nesslerization of perchloric acid filtrates.

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RECEIVED MARCH 5, 1956

SYNTHESIS OF COMPOUNDS RELATED TO RESERPINE. CONVERSION OF AN INTERMEDIATE TO 16-METHYLYOHIMBANE

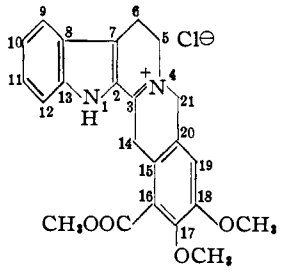
Sir:

In the course of investigating the relationship of structure to reserpine-like activity we have synthesized a number of derivatives related to reserpine having appropriate substituents at carbon atoms 16 and 18 of the yohimbane skeleton. We now wish to report the total synthesis of 16-carbomethoxy-18-hydroxy-$\Delta^{15(20)}$-yohimbene which contains a double bond at a position suitable for manipulating the stereochemistry of the important D/E ring junction.

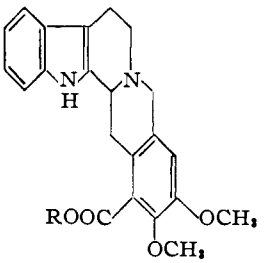
Treatment of methyl 2-carbomethoxy-3,4-dimethoxyphenylacetate[1] with chloromethyl ether and stannic chloride at 0° gave methyl 2-carbomethoxy-3,4-dimethoxy-6-chloromethylphenylacetate, m. p. 81–81.5° (found: C, 53.64; H, 5.41; Cl,

(1) C. Schöpf, U. Jäckh-Tettweiler, G. Mayer, H. Perrey-Fehrenbach and L. Winterhalder, *Ann.*, **554**, 77 (1940).

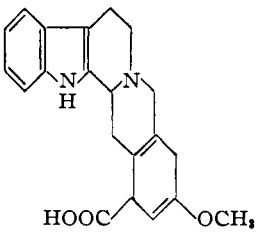
11.12). Condensation of this chloromethyl derivative with tryptamine in tetrahydrofuran at room temperature yielded N-(2-(3-indolyl)-ethyl)-3-oxo-5-carbomethoxy-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, m. p. 168–169° (found: C, 67.71; H, 5.73; N, 6.84). Cyclization of this lactam with phosphorous oxychloride at 100° furnished the unsaturated base (I), isolated as the hydrochloride, m. p. 245–247° (found: C, 65.13; H, 5.73; Cl, 8.38). The salt (I) was subsequently reduced with platinum in methanol to the saturated base (IIa), m.p. 205–207° (found: C, 70.55; H, 6.13; N, 7.07; eq. wt. (perchloric acid), 398). Hydrolysis of IIa with aqueous ethanolic potassium hydroxide gave the corresponding acid (IIb) (hydrochloride, m.p. 255–256°; found: C, 63.89; H, 5.58; N, 6.92; Cl, 8.20). Reduction of the acid with sodium in liquid ammonia in the presence of isopropylalcohol resulted in loss of the C-17 methoxyl group and further reduction to the enol ether (III) (m. p. 230–232°; found: C, 71.82; H, 6.35; λ_{max}^{Nujol} 5.85 μ, 6.00 μ)



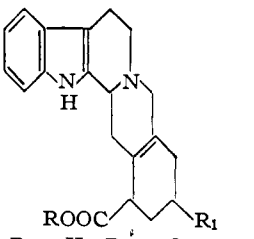
I



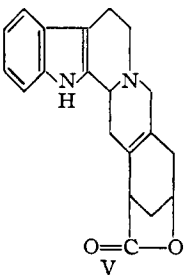
IIa, R = CH_3
IIb, R = H



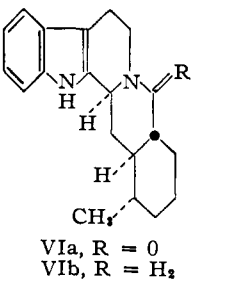
III



IVa, R = H; R_1 = O
IVb, R = H; R_1 = 
IVc, R = CH_3; R_1 = 

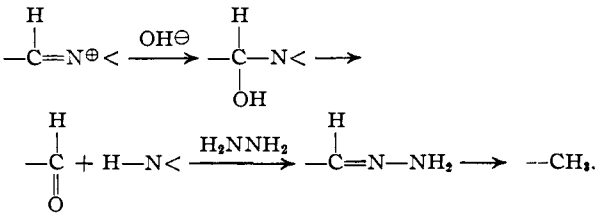


V



VIa, R = O
VIb, R = H_2

which on hydrolysis with dilute hydrochloric acid produced the unsaturated ketoacid (IVa), isolated as the hydrochloride, m. p. 251–254° ($C_{20}H_{20}O_3N_2 \cdot HCl \cdot 2H_2O$; found: C, 58.48; H, 6.44; N, 6.82; Cl, 8.68). The infrared spectrum of IVa showed carbonyl absorption at 5.84 μ but no absorption at 6.0 μ indicative of an α,β-unsaturated ketone. The double bond present in this ketoacid, therefore, must have remained in the unconjugated position between C_{15} and C_{20}. When IVa was reduced by the Wolff-Kishner method, a neutral lactam (VIa) ($C_{20}H_{24}ON_2$; m. p. 277–279°; found: C, 78.22, H, 7.60; N, 8.75; C—CH_3, 3.51; λ_{max}^{Nujol} 6.22 μ) was obtained. The formation of VIa is compatible with the presence of a C_{15}-C_{20} double bond since, presumably, the lactam is formed by migration of a double bond to the C_{21}-N_4 position followed by the reactions



Lactamization of the newly formed amine would then lead to VIa. The structure of the latter compound was proved by reduction with lithium aluminum hydride in tetrahydrofuran to give a basic compound (VIb), m. p. 191–193° ($C_{20}H_{26}N_2$; found: C, 81.75; H, 9.11; eq. wt. (perchloric acid), 292) which was shown to be *dl*-16-methylyohimbane by the identity of its infrared spectrum in carbon disulfide with that of anthentic 16-methylyohimbane (m. p. 193–195°) prepared by the method of Karrer and Saemann.[2]

Reduction of IVa with sodium borohydride gave a hydroxy acid (IVb), m. p. 255–256° (found: C, 71.02; H, 6.54) which formed a γ-lactone (V), m. p. 284–286° (found: C, 75.19; H, 6.37; λ_{max}^{Nujol} 5.65 μ) on treatment with pyridine and acetic anhydride. Since reduction of the aromatic carboxylic acid (IIb) by sodium in liquid ammonia should lead to the equatorially oriented carboxyl group, the formation of a γ-lactone indicates that the hydroxyl group at C_{18} in IVb is also equatorial as well as *cis* to the C_{16} carboxyl group. Treatment of the lactone (V) with sodium methoxide in methanol gave 18-hydroxy-16-carbomethoxy-$\Delta^{15(20)}$-yohimbene (IVc), m. p. 214–216° (found: C, 71.57; H, 6.74).

The pharmacological properties of these compounds will be reported elsewhere.

(2) P. Karrer and R. Saemann, *Helv. Chim. Acta*, **35**, 1932 (1952).

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THE REACTION OF RAUWOLFIA ALKALOIDS WITH MERCURIC ACETATE. CONVERSION OF 3-ISORESERPINE TO RESERPINE

Sir:

In conjunction with our work on the total synthesis of compounds related to reserpine,[1] a method was needed for converting an α-oriented C_3-hydrogen to the generally less stable β-oriented form.[2] We now wish to report a method by which this transformation may be accomplished.

(1) F. L. Weisenborn and H. E. Applegate, to be published.

(2) (a) E. Wenkert and L. H. Liu, *Experientia*, **11**, 302 (1955); (b) C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *ibid.*, **11**, 303 (1955).