TABLE II

SYNTHESIS OF CITRULLINE FROM CARBAMYL PHOSPHATE

The complete incubation mixture for the carbamyl phosphate system contained: 200 μM. tris-(hydroxymethyl)-aminomethane buffer, *p*H 8.5; 10 μM. ornithine, *p*H 8.0; 5 μM. $MgCl_2$; 6.2 μM. CAP (containing 0.8 μM. orthophosphate); and 0.1 mg. protein as the *Streptococcus faecalis* extract in 1 ml. final volume. The vessels were incubated at 30° for 30 minutes.

	P_i, μM./ml.	CAP, μM./ml.	Citrulline, μM./ml.
Complete, zero time	0.8	6.20	0
Complete, incubated	6.9	0	6.3
No Mg	6.8	0	6.3
No ornithine	2.4	4.7	0
No enzyme	2.3	4.5	0

TABLE III

FORMATION OF ATP FROM CARBAMYL PHOSPHATE

The complete incubation mixture contained: 200 μM. tris-(hydroxymethyl)-aminomethane buffer, *p*H 8.5; 10 μM. $MgCl_2$; 7.20 μM. of ADP, *p*H 7.0; 8.58 μM. of CAP (containing 1.3 μM. inorganic phosphate and 1.5 μM. ammonia[a]); 0.5 mg. protein as the *Streptococcus faecalis* extract in 1 ml. final volume. Vessels were incubated at 30° for 20 minutes. The difference figures represent the difference between the complete system deproteinized at zero time and the reaction vessels.

	P_i, μM./ml.	ATP, P_{10}, μM./ml.	CAP P_U, μM./ml.	CAP NH_3,[a] μM./ml.
Complete	+0.30	+7.4	−7.35	+7.5
No enzyme	+1.20	0	−1.64	0
No ADP	+1.30	0	−1.32	...
No Mg	+0.86	+3.4	−4.54	+3.2

[a] Ammonia was determined by a Conway distillation procedure[10] using saturated K_2CO_3 to liberate the ammonia from solution. The data reflect the stability of the fixed carbamate to alkali (*cf.* ref. 11).

experiment of Table IV, a rapid transfer from CAP is shown to occur while in the same extract, not shown in the table, ATP was inactive. With intact mitochondria, ATP showed in 60 minutes only about one-fifth of the activity shown by CAP in 10 minutes. With similar preparations, Grisiolia and Cohen[12] have previously reported on an unstable precursor of the carbamyl group in citrulline.

TABLE IV

Sonorated extract of rat liver mitochondria, 0.1 ml. (0.9 mg. protein), in 1 ml. total volume, *p*H 7.5, incubated 30 minutes at 37°. Otherwise conditions were similar to those in Table II.

	P_i, μM./ml.	CAP, μM./ml.	Citrulline, μM./ml.
Complete, 0 minutes	1.0	4.2	0
Complete, incubated	5.2	0	4.1
No CAP, incubated	0.1	0	0

If aspartic acid is substituted for ornithine in the microbial extracts as carbamyl acceptor, a somewhat slower reaction with carbamyl phosphate is observed, indicating an analogous mechanism for the synthesis of carbamyl aspartate. Formation of this compound from aspartate with ATP and ammonium carbonate or an unstable carbamyl precursor has recently been described in mammalian liver extracts by Lowenstein and Cohen[13] and by P. Reichard.[14]

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CANESCINE AND PSEUDOYOHIMBINE FROM THE ROOTS OF *RAUWOLFIA CANESCENS* L.[1]

Sir:

Four alkaloids, α-yohimbine (rauwolscine),[2] yohimbine,[3] serpentine[3] and reserpine,[4] have so far been isolated from the roots of *Rauwolfia canescens* L. Reserpine was first discovered in *Rauwolfia serpentina*, and the hypotensive and sedative effects of rauwolfia have been mainly attributed to it.

We have now isolated two further alkaloids in pure form from the methanol mother liquor of reserpine, obtained from roots of *Rauwolfia canescens*,[5] by means of chromatography with aluminum oxide and fractional crystallization.

One of the two alkaloids, which crystallizes from methanol in hexagonal plates free from solvent, has been found to be identical with pseudoyohimbine which was originally found in yohimbé bark,[6] m.p. 265–278° (cor., in vacuum tube) with decomposition,[7] $[\alpha]^{20}D$ +27 ± 2° (*c* 0.3 in pyridine); $C_{21}H_{26}O_3N_2$ (354.4) (calcd.: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.08; H, 7.56; N, 8.21). Molecular weight determined by potentiometric titration with 0.1 *N* HCl was 358. The hydrochloride crystallizes from alcohol in needles containing solvent, m.p. 250–260 (cor.) with decomposition, $C_{21}H_{26}O_3N_2 \cdot HCl$ (calcd.: C, 64.52; H, 6.96; Cl, 9.07. Found: C, 64.52; H, 7.02; Cl, 9.66). In Keller's color reaction with glacial acetic acid containing ferric chloride, and concentrated H_2SO_4, the alkaloid yields a brownish-violet stain like that of yohimbine. The ultraviolet spectrum in ethanol reveals maxima at 226 mμ and 280 mμ and a small peak at 291 mμ.

For the second alkaloid, which is not identical with any known compound, we propose the name *canescine*. It crystallizes from 15 parts of methanol

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(7) Our thanks are due to Prof. M. M. Janot and Dr. R. Goutarel, of Paris, for making available an authentic sample of pseudoyohimbine and for verifying the identity of our sample of pseudoyohimbine.

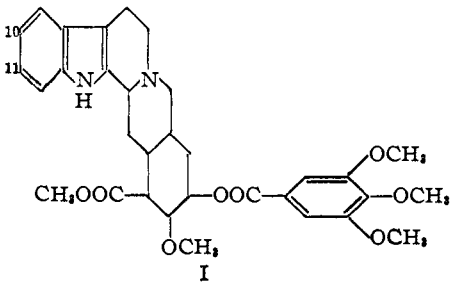
in thick pentagonal and hexagonal plates which melt in the capillary tube at 230–234° (cor.) with decomposition, $[\alpha]_D$ −163 ± 2° (*c* 0.5 in pyridine). The values obtained on analysis indicate the empirical formula $C_{32}H_{38}O_8N_2$ (578.6) (calcd. C, 66.42; H, 6.62; O, 22.12; N, 4.84. Found: C, 66.43; H, 6.51; O, 22.14; N, 4.91). The molecular weight determined by potentiometric titration with 0.1 *N* HCl was 579. The hydrochloride crystallizes from watery acetone in thin, rectangular plates, m.p. 247–253° (cor.) with decomposition; $C_{32}H_{38}O_8N_2 \cdot HCl$ (calcd. C, 62.48; H, 6.39; O, 20.81; Cl, 5.76. Found: C, 62.09; H, 6.15; O, 20.93; Cl, 5.86). In Keller's color reaction with glacial acetic acid containing ferric chloride, and concentrated sulfuric acid, canescine yields the same brownish-violet stain as yohimbine.

On alkaline hydrolysis, canescine yields an equivalent of trimethoxybenzoic acid, $C_{10}H_{12}O_5$ (212.2) (calcd.: C, 56.60; H, 5.70; O, 37.70. Found: C, 57.13; H, 5.90; O, 37.03), m.p. and m.p. when mixed with a genuine sample of trimethoxybenzoic acid were 170° (cor.)

If canescine is boiled with sodium methylate in methanol, canescinic acid methyl ester and trimethoxybenzoic acid methyl ester are obtained. Canescinic acid methyl ester has not yet been obtained in crystalline form; $C_{22}H_{28}O_4N_2$ (384.5) (calcd. C, 68.72; H, 7.34; O, 16.65. Found: C, 68.02; H, 7.41; O, 16.93), $[\alpha]^{20}_D$ −80 ± 2° (*c* 0.5 in pyridine).

The ultraviolet absorption spectrum of canescine in ethanol is composed of the chromophores of the unsubstituted indole system and of trimethoxybenzoic acid; λ_{max} 218 (log ε 4.79), λ_{max} 272 (log ε 4.26), λ_{max} 290 (log ε 4.07).

From these data and from biogenetic considerations it may be concluded, with a high degree of probability, that canescine has the structure of a 11-desmethoxyreserpine (formula I).



I

Investigations have shown that canescine possesses pharmacological properties similar to those of reserpine; above all, it produces a marked and prolonged fall in blood pressure.[8] The methoxy group in position 11 of the reserpine molecule, which is absent in canescine, therefore, does not seem to be necessary for the pharmacodynamic actions typical of these substances.

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YEAST ALCOHOL DEHYDROGENASE, A ZINC METALLOENZYME

Sir:

Crystalline alcohol dehydrogenase (ADH) of yeast[1] is a zinc metalloenzyme; zinc is an integral and enzymatically functional component of the apoenzyme molecule.

Qualitative and quantitative emission spectrography was performed in duplicate,[2,3] as were microchemical determinations of zinc.[4,5] Protein weights were determined by trichloroacetic acid precipitation[6] and separately by measurement of absorbance at λ 280 mμ.

Crystalline preparations from our own and commercial sources uniformly contained large quantities of zinc, lesser and variable quantities of magnesium and insignificant amounts of all other elements.

Table I gives typical, quantitative spectrochemical data on twice crystallized preparations of yeast ADH, having high activity. Preparation 1 contained 1660 μg. of zinc per gram of protein, preparation 2 contained 1440 μg. per gram. Fractionation of ADH demonstrated an increase in the zinc:protein ratios in fractions in which the activity:protein ratio was increased. The metal:

TABLE I

EMISSION SPECTROGRAPHIC ANALYSIS OF TWICE CRYSTALLIZED YEAST ALCOHOL DEHYDROGENASE

Element	Preparation no. 1 Line/internal standard	Preparation no. 1 μg./g. yeast ADH	Preparation no. 2 Line/internal standard	Preparation no. 2 μg./g. yeast ADH
Zinc	Zn 3345 / Bi 2897	1660	Zn 3345 / V 3185	1440
Copper	By Na Diethyldithiocarbamate	165		[a]
Iron	Fe 3020 / V 3185	80	Fe 4283 / V 4395	81
Aluminum	Al 3961 / V 3185	79	Al 3961 / V 3185	48
Magnesium	Mg 2776 / Bi 2897	1180	Mg 2798 / V 3185	296
Calcium	Ca 4318 / Bi 2897	39	Ca 4302 / V 4395	105
Strontium	Sr 4077 / V 4111	4	Sr 4077 / V 4395	2
Barium	Ba 4554 / V 3185	11	Ba 4554 / V 4395	20
Manganese	Mn 2576 / V 4111	2	Mn 4030 / V 4395	π
Lead	Pb 4057 / V 4111	45	Pb 4057 / V 4395	π
Cadmium	Cd 2268 / V 3185	13	...	π
Chromium	Cr 4254 / V 4111	8	Cr 4254 / V 4395	π

Not detected: π, and also beryllium, cobalt, lithium, molybdenum, nickel, potassium, silver, tin.

[a] Lost.

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