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BIOGENESIS OF THE RAUWOLFIA ALKALOIDS—II

THE INCORPORATION OF TRYPTOPHAN INTO SERPENTINE AND RESERPINE

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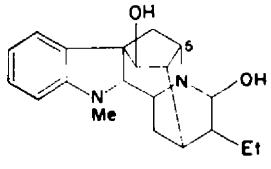
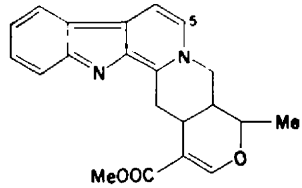
Abstract—Radioactive serpentine which was obtained from *Rauwolfia serpentina* plants which had been fed tryptophan-2-C^{14} was degraded systematically and was found to be labeled solely at C-5. This result indicates that tryptophan is a direct precursor of the β-carboline moiety of this alkaloid. Radioactive reserpine which was obtained from the same feeding experiment was labeled only on the reserpic acid part of the alkaloid.

WE have previously reported[1] that the administration of DL-tryptophan-2-C^{14} to *R. serpentina* plants (3 years old) led to the formation of radioactive ajmaline (I), serpentine (II) and reserpine (III). We chose to degrade the ajmaline first since it is the most abundant alkaloid in *R. serpentina* and had the highest specific activity.



I



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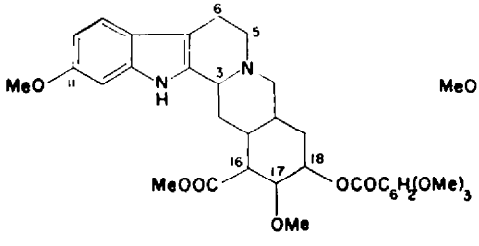
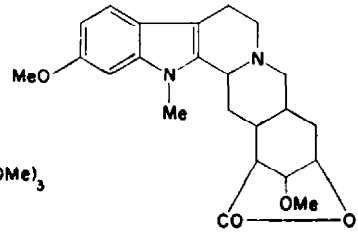


III



IV

Our results[1] indicated conclusively that the ajmaline was labeled solely at C-5. This result was in agreement with previously conceived hypotheses on the biogenesis of ajmaline.[2,3] It was a reasonable assumption that serpentine and reserpine would also be specifically labeled at C-5 of their β-carboline moieties. Therefore our initial experiments were directed towards the conversion of these alkaloids to simple derivatives of harman which could be degraded by the procedure previously worked out for the

[1] E. Leete, *J. Amer. Chem. Soc.* **82**, 6338 (1960).
[2] R. B. Woodward, *Angew. Chem.* **68**, 13 (1956).
[3] R. Robinson, *Festschrift Arthur Stoll* p. 457. Birkhäuser, Basel (1957).

degradation of the radioactive ajmaline. Ajmaline yielded N-(ind)-methylharman (VI) on heating with sodalime at 360°,[4] but serpentine failed to yield any harman under similar conditions. However since Leonard and Elderfield[5] obtained harman by the fusion of alstonine with potassium hydroxide, it seemed reasonable to expect that the same compound would be obtained from serpentine since the two alkaloids are stereoisomers. This proved to be the case, a 20 per cent yield of harman being obtained. The harman which had the same specific activity as the serpentine was methylated according to the procedure of Potts and Saxton[6] to yield N-(ind)-methylharman. Subsequent degradation proceeded as illustrated in Fig. 1 and is identical with that used for the N-(ind)-methylharman derived from the radioactive ajmaline. In our earlier

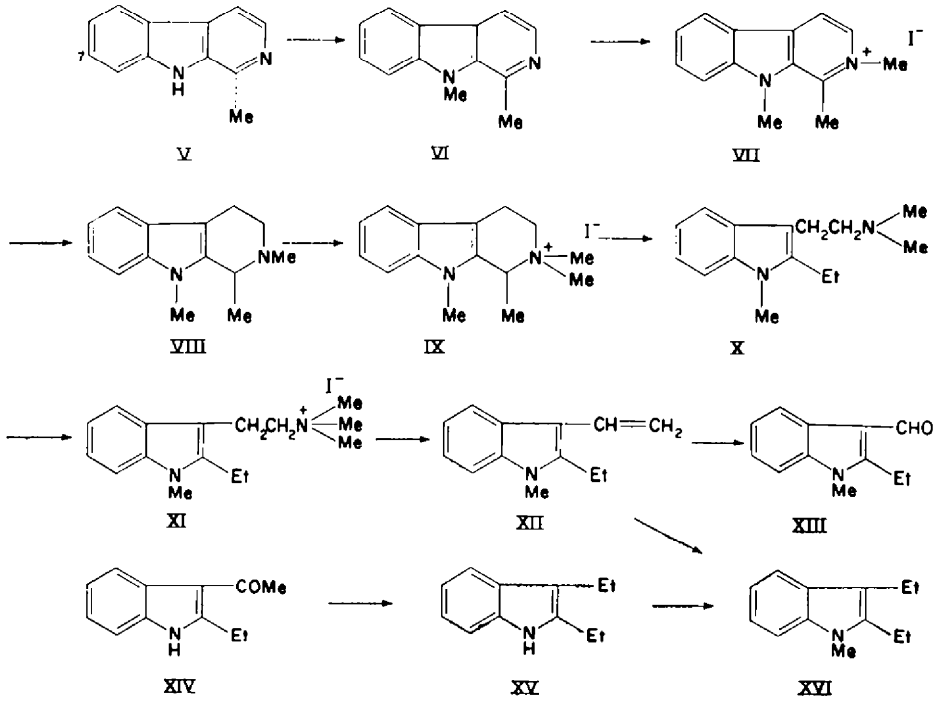


FIG. 1. Degradation of the harman derived from serpentine and related reactions.

paper[1] we made no attempt to characterize the 2-ethyl-1-methyl-3-vinyl-indole (XII) obtained by the Hofmann degradation of 2-ethyl-1-methyl-N, N-dimethyltryptamine methiodide (XI). Its structure was assigned on the basis of its mode of formation and its subsequent oxidation with osmium tetroxide and periodate to 2-ethyl-1-methyl-3-indole aldehyde and formaldehyde. We have now found that a freshly prepared sample analyzed correctly and yielded a stable 1,3,5-trinitrobenzene derivative. The ultraviolet spectrum of theis 3-vinyl indole is illustrated in Fig. 2, together with its hydrogenation product, 2,3-diethyl-1-methyl-indole. The pronounced absorption at 265 mμ is thus assignable to the 3-vinyl indole chromophor. It is of interest to note that this absorption had decreased 50 per cent in a sample of the vinylindole

[4] F. A. L. Anet, D. Chakravarti, R. Robinson and E. Schlittler, *J. Chem. Soc.* 1242 (1954).
[5] N. J. Leonard and R. C. Elderfield, *J. Org. Chem.* 7, 556 (1942).
[6] K. T. Potts and J. E. Saxton, *J. Chem. Soc.* 2641 (1954).

which was 12 hours old, presumably indicating extensive polymerization. 2,3-Diethyl-1-methylindole (XVI) was prepared for comparison with the hydrogenation product of the vinylindole as follows. 2-Ethylindole was refluxed with a mixture of acetic acid and acetic anhydride to yield 3-acetyl-2-ethylindole (XIV). This ketone was reduced[7] with

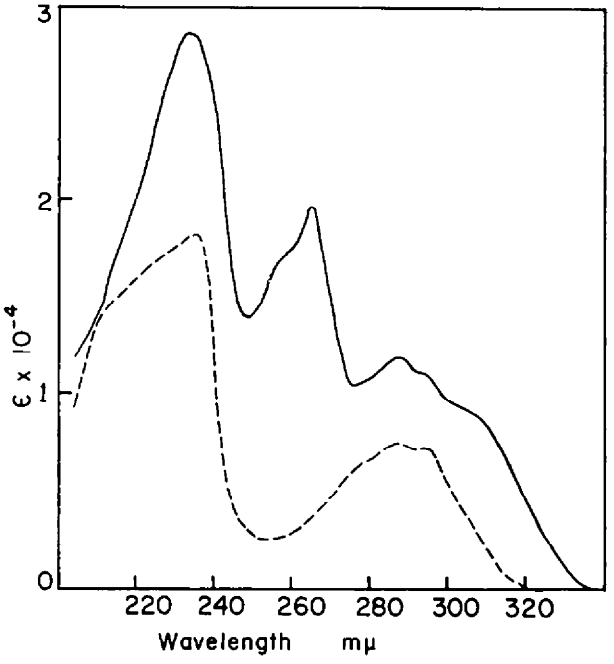


FIG. 2. Ultraviolet spectra in 95% ethanol of 2-ethyl-1-methyl-3-vinylindole (XII) [full line] and 2,3-diethyl-1-methyl indole (XVI) [broken line].

lithium aluminum hydride to 2,3-diethylindole which was methylated[6] to yield XVI. The activities of serpentine and its degradation products are recorded in Table 1. It is seen that the 2-ethyl-1-methyl-3-indole aldehyde was inactive whereas all the other degradation products had essentially the same specific activity as the serpentine indicating that all the activity was located at C-5 of the serpentine molecule. This result strongly suggests that tryptophan is a direct precursor of the β-carboline moiety of this alkaloid, in agreement with previous hypotheses.[8]

Our attempts to convert reserpine to simple derivatives of harman or harmine (7-methoxyharman) have not so far been successful. Basic hydrolysis of the radioactive reserpine yielded 3,4,5-trimethoxybenzoic acid which was completely inactive and reserpic acid which had the same specific activity as the reserpine. Fusion of reserpic acid with potassium hydroxide or heating with sodalime failed to yield any harmine. When reserpine was dissolved in liquid ammonia containing sodamide and treated with methyl iodide two compounds were isolated from the reaction mixture, 3,4,5-trimethoxybenzamide and a compound melting at 219–220°. This compound had no absorption in the NH region of the infrared spectrum but had a strong absorption at 1775 cm^{-1} assignable to a carbonyl group in a γ-lactone. The properties of

[7] *cf.* E. Leete and L. Marion, *Canad. J. Chem.* **31**, 775 (1953).
[8] *cf.* R. Robinson, *The Structural Relations of Natural Products* p. 100. Clarendon Press, Oxford (1955).

this compound are consistent with formula IV, i.e. N-(ind)-methylreserpic acid lactone. The formation of these two compounds is readily rationalized. Attack by amide ions on the ester carbonyl at C-18 yields 3,4,5-trimethoxybenzamide and generates an alkoxide ion which attacks the ester carbonyl at C-16 eliminating an ethoxide ion and

TABLE 1. ACTIVITIES OF SERPENTINE, RESERPINE AND THEIR DEGRADATION PRODUCTS

	Activity* (counts/min per mmole)
Serpentine (II)	$1{\cdot}2 \times 10^6$
Serpentine nitrate	$1{\cdot}1 \times 10^6$
Harman (V)	$1{\cdot}1 \times 10^6$
N-(ind)-Methyl-1,2-dimethyl-1,2,3,4-tetrahydro-β-carboline-N$_\beta$-methiodide (IX)	$0{\cdot}9 \times 10^6$
2-Ethyl-1-methyl-N, N-dimethyltryptamine methiodide (XI)	$1{\cdot}2 \times 10^6$
2-Ethyl-1-methyl-3-indole aldehyde (XIII)	0
Formaldehyde dimedone derivative	$1{\cdot}1 \times 10^6$
Reserpine (III)	$5{\cdot}4 \times 10^5$
Reserpine perchlorate	$5{\cdot}5 \times 10^5$
Reserpic acid hydrochloride	$5{\cdot}3 \times 10^5$
3,4,5-Trimethoxybenzoic acid	0

* Radioactivity determinations were carried out in a Nuclear-Chicago Model D-47 Q-gas flow counter using a micromil window. Determinations were carried out on samples of finite thickness, making corrections for efficiency and self absorption.

producing the γ-lactone. Methylation of the indolic nitrogen then yields IV. Fusion of the lactone IV with potassium hydroxide failed to yield any N-(ind)-methylharmine.

Reserpine was dehydrogenated to 3,4,5,6-tetradehydroreserpine with maleic acid in the presence of palladium.[9] However, potassium hydroxide fusion of the tetradehydroreserpic acid obtained by hydrolysis of this compound failed to yield any harmine.

It is of interest to compare the incorporation of radioactivity into the alkaloids of *R. serpentina*. One common method of making such a comparison is by means of the "Percentage Incorporation". This is the total activity found in the alkaloid divided by the total activity fed to the plant. This value is not useful as a basis for comparison in this case since the alkaloids were not recovered quantitatively from the plant and they occur in unequal amounts. It is more meaningful to compare the "Dilution", which is the specific activity of the adminstered tryptophan-2-C^{14} divided by the specific activity of the isolated alkaloid. These results are recorded in Table 2. It is seen that the greatest dilution has occurred with the reserpine. There are many possible explanations for this result and a great deal more work will have to be done before we can account for this. Thus one would have to carry out feeding experiments with plants of different ages in order to determine whether the alkaloids are being produced at different rates at different stages in the development of the plant. The lower incorporation into reserpine could also be related to the presence of the methoxyl group at C-11,

[9] E. Wenkert, E. W. Robb and N. V. Bringi, *J. Amer. Chem. Soc.* **79**, 6570 (1957); *cf.* also *Ibid.* **81**, 2494 (1959).

which is absent in ajmaline and serpentine. At this time we have no information on the mechanism of hydroxylation at this position, and we do not know at which stage in the biogenesis of reserpine this occurs. It would be of interest to isolate deserpidine (reserpine lacking the 11-methoxyl group) and compare its activity with that of

TABLE 2. COMPARISON OF THE INCORPORATION OF TRACER INTO THE ALKALOIDS OF *R. serpentina*

	Weight (mg)	Activity (counts/min per mmole)	Percentage incorporation	Dilution
Ajmaline	80	$2{\cdot}5 \times 10^6$	0·25	710
Reserpine	44	$5{\cdot}4 \times 10^5$	0·018	3300
Serpentine	5·7*	$1{\cdot}2 \times 10^6$	0·007	1500

* Isolated as the nitrate.

reserpine. Questions such as these will occupy the attention of those studying the biogenesis of alkaloids and other natural products for many years to come.

EXPERIMENTAL

Melting points are corrected. The petroleum ether had b.p. 60–80°.

Degradation of the radioactive serpentine

The radioactive serpentine nitrate (5·7 mg) which had been isolated from the *R. serpentina* plants, which had been fed DL-tryptophan-2-C^{14} (37·5 mg, total activity $2{\cdot}2 \times 10^8$ counts/min), was mixed with inactive serpentine hydrochloride (472 mg), dissolved in hot water and neutralized with sodium carbonate. The yellow precipitate of serpentine was crystallized from ethanol until it had a constant specific activity. A small sample was converted to the nitrate as a check on its radiochemical purity.

The diluted serpentine (333 mg) was powdered in a mortar with potassium hydroxide (8·0 g). The intimate mixture was fused in a nickel crucible and heated with stirring in an atmosphere of nitrogen in a metal bath maintained at 360° for 40 min. The contents of the crucible were allowed to cool and ice added. The dark brown solution was extracted several times with ether. The dried ether extract was evaporated to small bulk and ethanolic hydrochloric acid added when a pale brown precipitate of crude harman hydrochloride (100 mg) separated. This hydrochloride was dissolved in water, made basic with sodium hydroxide and extracted with benzene. The dried benzene solution was chromatographed on alumina (activity III) eluting first with benzene and then with 10% ethanol in benzene when the harman was obtained. The harman was further purified by sublimation (200°, 0·001 mm) and the pale yellow sublimate crystallized from a mixture of benzene and petroleum ether to yield harman (34 mg, 20% yield), m.p. 237–238° identical (I.R. spectrum and mixed m.p.) with authentic material. The harman was converted to N-(ind)methylharman by dissolving in liquid ammonia containing sodamide and then adding methyl iodide.[6] The degradation then proceeded through to 2-ethyl-1-methyl-3-indolealdehyde (XIII) and the dimedone derivative of formaldehyde as described in Part I of this series.[1] The specific activity of serpentine and its degradation products are recorded in Table 1 and are calculated for carrier-free material.

2-Ethyl-1-methyl-3-vinylindole (XII)

This vinylindole was obtained as previously described[1] from 2-ethyl-1-methyl-N, N-dimethyltryptamine methiodide. The freshly prepared and redistilled vinylindole was obtained as a colorless liquid (Found: C, 84·20; H, 8·15; N, 7·84. $C_{13}H_{15}N$ requires: C, 84·28; H, 8·16; N, 7·56%). The ultraviolet spectrum in 95% ethanol is recorded in Fig. 2. Its infrared spectrum as a liquid film had a strong absorption at 1625 cm^{-1} which may be assigned to the stretching vibration of the vinyl group since this absorption is absent in its hydrogenation product, 2,3-diethyl-1-methylindole. The

1,3,5-*trinitrobenzene complex of* 2-*ethyl*-1-*methyl*-3-*vinylindole* was obtained from methanol as violet-brown prismatic needles, m.p. 135–136° (Found: C, 57·33; H, 4·64. $C_{19}H_{18}O_6N_4$ requires: C, 57·28; H, 4·55%).

2,3-*Diethyl*-1-*methylindole* (XVI)

(a) *From* 2-*ethyl*-1-*methyl*-3-*vinylindole*. The vinylindole (80 mg) was dissolved in absolute ethanol (50 ml) and hydrogenated at 2 atm press in the presence of platinum oxide (40 mg) for 2 hr. The filtered solution was evaporated and the residue distilled *in vacuo* (160°, 0·01 mm) to yield 2,3-*diethyl*-1-*methyl-indole* as a colorless oil, n_D^{27} 1·5724 (Found: C, 83·12; H, 9·15; N, 7·30. $C_{13}H_{17}N$ requires: C, 83·37; H, 9·15; N, 7·48%). Its ultraviolet spectrum in 95% ethanol is recorded in Fig. 2. The 1,3,5-*trinitrobenzene complex of* 2,3-*diethyl*-1-*methylindole* was obtained as bright red prismatic needles from methanol, m.p. 84–85° (Found: C, 56·89; H, 5·15; N, 13·86. $C_{19}H_{20}O_6N_4$ requires: C, 56·99; H, 5·04; N, 13·99%).

(b) *From* 2-*Ethylindole: 3-Acetyl-2-ethylindole*. 2-Ethylindole[10] (30 g) was refluxed in a nitrogen atmosphere with a mixture of acetic acid (20 ml) and acetic anhydride (150 ml) for 20 hr. The bulk of the solvent was removed under reduced pressure and the dark brown residue diluted with ethanol when the crude product separated out (15 g). Several crystallizations from ethanol (charcoal) yielded 3-acetyl-2-ethylindole as small colorless prisms, m.p. 159–160° (Found: C, 77·17; H, 7·17; N, 7·62. $C_{12}H_{13}ON$ requires: C, 76·97; H, 7·01; N, 7·48%). Its infrared spectrum (KBr pellet) had a carbonyl absorption at 1627 cm^{-1}.

2,3-*Diethylindole*. 3-Acetyl-2-ethylindole (1·87 g) was suspended in ether (250 ml) and a solution of 4% lithium aluminum hydride in ether (30 ml) added and the mixture refluxed for 20 hr. Water was then added to the cooled mixture which was filtered, dried and evaporated. The residue was distilled (160° 0·01 mm) to yield 2,3-*diethylindole* (1·56 g) as a colorless oil having a strong violet fluorescence, n_D^{27} 1·5829.[11] On prolonged cooling the liquid solidified to a colorless crystalline solid, m.p. 29–30° (Found: C, 82·96; H, 8·62; N, 8·25. $C_{12}H_{15}N$ requires: C, 83·19; H, 8·73; N, 8·09%). The 1,3,5-*trinitrobenzene complex of* 2,3-*diethylindole* was obtained as brownish red prismatic needles from methanol, m.p. 135–136° (Found: C, 55·78; H, 4·65; N, 14·70. $C_{18}H_{18}O_6N_4$ requires: C, 55·95; H, 4·70; N, 14·50%). The *picrate of* 2,3-*diethylindole* was obtained as dark brown prisms from ethanol, m.p. 135–136° (Found: C, 53·87; H, 4·57; N, 13·83 $C_{18}H_{15}O_7N_4$ requires: C, 53·73; H, 4·51; N, 13·93%).

2,3-*Diethyl*-1-*methylindole*. Sodium (150 mg) was dissolved in liquid ammonia (200 ml) containing a small crystal of ferric nitrate. When the blue color had disappeared 2,3-diethylindole (665 mg) dissolved in liquid ammonia was added and an orange solution was obtained. Methyl iodide (0·5 ml) was added and the ammonia allowed to evaporate. Water was added to the residue and the mixture extracted with ether. The dried ether extract was evaporated and distilled (160°, 0·01 mm) to yield 2,3-diethyl-1-methylindole, having an infrared spectrum identical with the material obtained from the vinylindole XII.

Degradation of the radioactive reserpine

(a) *Hydrolysis*. The active reserpine (44 mg) was diluted with inactive material (300 mg) and crystallized from a mixture of methanol and acetone until it had a constant specific activity. A further check on its radiochemical purity was obtained by the preparation of reserpine perchlorate. The diluted reserpine was hydrolysed with sodium hydroxide in aqueous methanol according to established methods,[12] yielding 3,4,5-trimethoxybenzoic acid and reserpic acid which was isolated as its hydrochloride. The activity of reserpine and its degradation products are recorded in Table 1.

(b) *Attempted* N-*methylation of reserpine*. Reserpine (300 mg) was added to a solution of sodium (40 mg) in liquid ammonia (200 ml). When all the reserpine had dissolved methyl iodide (0·1 ml) was added and the ammonia then allowed to evaporate. Water was added to the residue and the mixture

[10] A. Verley and J. Beduwé, *Bull. Soc. Chim. Fr.* [4] **37**, 190 (1925).

[11] V. F. Martynov and V. F. Martynova [*Zh. Obshchei Khim.* **24**, 2146 (1954); *Chem. Abstr.* **50**, 287 (1956)] describe 2,3-diethylindole as a liquid having n_D^{20} 1·5806 and affording a picrate, m.p. 121–122°. The product obtained by A. E. Arbuzov and I. A. Zaitzev [*Trans. Butlerov Inst. Chem. Tech. Kazan* No. 1, 33 (1934); *Chem. Abstr.* **29**, 4006 (1935)] by the cyclization of the phenylhydrazone of ethylpropylketone was either 2,3-diethylindole or 3-methyl-2-propylindole. Since their compound yielded a picrate, m.p. 144°, it seems likely that the latter indole was obtained.

[12] N. Neuss, H. E. Boaz and J. W. Forbes, *J. Amer. Chem. Soc.* **76**, 2463 (1954).

extracted with chloroform. The dried chloroform extract was evaporated and the residue dissolved in ethanol (2 ml), diluted with benzene (20 ml) and chromatographed on alumina (activity III). Elution with benzene containing 10% ethanol yielded a pale yellow solid which on crystallization from benzene-petroleum ether gave small colorless prisms (70 mg) of N-(*ind*)-*methylreserpic acid lactone* (IV), m.p. 219–220° (Found: C, 69·28; H, 7·18; N, 7·09. $C_{23}H_{28}O_4N_2$ requires: C, 69·67; H, 7·12; N, 7·07%). Its infrared spectrum had no absorption in the NH region, but had a strong absorption at 1775 cm^{-1} assignable to the carbonyl in a γ-lactone. Further elution of the alumina column with absolute ethanol yielded 3,4,5-trimethoxybenzamide (25 mg), m.p. 182–183°, identical (I.R. spectrum and mixed m.p.) with an authentic specimen. (Found: C, 56·54; H, 6·31; N, 6·67. Calc. for $C_{10}H_{13}O_4N_2$: C, 56·86; H, 6·20; N, 6·63%).

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