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The Biogenesis of the Rauwolfia Alkaloids. I. The Incorporation of Tryptophan into Ajmaline[1]

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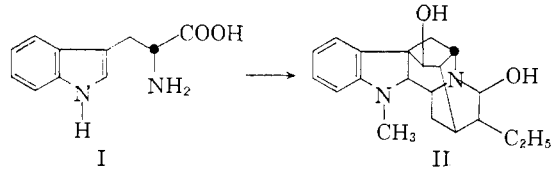
RECEIVED MAY 28, 1960

The biogenesis of the indole alkaloids found in *Rauwolfia serpentina* has been investigated by the use of radioactive tracers. When DL-tryptophan-2-C^{14} was injected into the stems of intact plants there was a significant incorporation of tracer into ajmaline, reserpine and serpentine. Systematic degradation of the radioactive ajmaline indicated that all the activity was located on one carbon atom and established that tryptophan is a precursor of the reduced β-carboline moiety of this alkaloid.

The alkaloids found in *Rauwolfia* species have received widespread attention in recent years by chemists, pharmacologists and physicians.[2] The most important alkaloid, from a medical point of view, is reserpine which is used for the treatment of hypertensive, nervous and mental disorders. Two other alkaloids, ajmaline and serpentine are fairly abundant in *R. serpentina* Benth, the species which has been investigated most extensively.

All are agreed that tryptophan or tryptamine is a likely precursor of the β-carboline moiety of these alkaloids. Thus tryptophan-2-C^{14} (I) would be expected to yield ajmaline labeled as indicated in formula II. The origin of the rest of the molecule



I II

is the subject of controversy. On one side it has been suggested that the E ring of reserpine, which is considered to exist in a fragmented form in ajmaline and serpentine, arises from the benzene ring of 3,4-dihydroxyphenylalanine.[3,4] Wenkert has proposed an alternative hypothesis[5] whereby the intact or modified E ring of these alkaloids is derived from shikimic acid.

In our preliminary experiments we administered radioactive compounds to *R. serpentina* by allowing the roots of the intact plants to grow in a nutrient solution containing the tracer. In separate experiments we fed tryptophan-3-C^{14}, tyrosine-2-C^{14} and 6-methoxytryptophan-3-C^{14} to the plants. The alkaloids isolated several weeks later were either inactive or of very low activity. It was observed that the tryptophan and 6-methoxytryptophan were absorbed very slowly from the nutrient solution and these negative results may simply indicate that the radioactive amino acids were not reaching the site of alkaloid synthesis. In a later experiment tryptophan-2-C^{14} was injected directly into the stems of the plant (see Experimental for details), and in this case a significant amount of activity was found in the ajmaline, reserpine and serpentine isolated from the plant 45 days after the initial feeding. Since the ajmaline had the highest specific activity and was the most abundant alkaloid, we selected it for degradation to determine the location of the radioactive carbon in the molecule.

Ajmaline yields N-(ind)-methylharman (III) on heating with soda-lime,[6] and this proved to be a convenient starting material for the systematic degradation of the radioactive ajmaline. Furthermore this compound was readily prepared from tryptophan or N-(ind)-methyltryptophan and was thus available in quantity for working out the degradative scheme shown in Fig. 1. N-(ind)-Methylharman was converted to its methiodide (IV) which was reduced with sodium borohydride in ethanol solution to N-(ind)-methyl-1,2-dimethyl-1,2,3,4-tetrahydro-β-carboline (V). Emde reduction (sodium in liquid ammonia) on the methiodide of this tertiary base (VI) gave a single compound in high yield. By analogy with the Emde reduction of tetrahydroisoquinolines[7,8] the expected product was 2-ethyl-1-methyl-N,N-dimethyltryptamine (VII). This was established by an unambiguous synthesis of its methiodide (VIII). 2-Ethylindole (XI) reacted smoothly with nitroethylene[9] to yield 2-ethyl-3-(2-nitroethyl)-indole (XII) which was reduced catalytically to 2-ethyltryptamine (XIII). This was methylated on the indole nitrogen using the procedure of Saxton[10] to yield the 2-ethyl-1-methyltryptamine (XIV). Further reaction of this compound with methyl iodide in the presence of sodium bicarbonate afforded VIII, identical with the substance derived from N-(ind)-methylharman. Hofmann degradation of the methiodide VIII gave 2-ethyl-1-methyl-3-vinylindole (IX) in excellent yield. This compound had an absorption maximum in the ultraviolet at 265 mμ (ϵ = 20,000) with an inflection at 258 mμ. An absorption in this region of the spectrum seems to be a characteristic of 3-vinylindoles[11]

(1) Part of this work has been reported briefly in a communication: E. Leete, *Chem. and Ind. (London)*, 692 (1960), and was presented in a lecture delivered at the 7th National Medicinal Chemistry Symposium of the American Chemical Society, Kingston, Rhode Island, June 20–22, 1960. This investigation was supported by a research grant MY 2662, from the National Institute of Mental Health, Public Health Service.

(2) A. Chatterjee, *Fortschr. Chem. Org. Naturst.*, **10**, 390 (1953); A. Chatterjee, S. C. Pakrashi and G. Werner, *ibid.*, **13**, 346 (1956); "Rauwolfia: Botany, Pharmacognosy, Chemistry and Pharmacology," by R. E. Woodson, H. W. Youngken, E. Schlittler and J. A. Schneider, Little Brown and Co., Boston, Mass., 1957; R. Robinson in "Festschrift Arthur Stoll," Birkhauser, Basel, 1957, p. 457; J. J. Lewis, *J. Pharm. Pharmacol.*, **8**, 465 (1956).

(3) R. Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p. 100.

(4) R. B. Woodward, *Angew. chem.*, **68**, 13 (1956).

(5) E. Wenkert and N. V. Bringi, THIS JOURNAL, **81**, 1474 (1959).

(6) F. A. L. Anet, D. Chakravarti, R. Robinson and E. Schlittler, *J. Chem. Soc.*, 1242 (1954).

(7) D. B. Clayson, *ibid.*, 2016 (1949).

(8) G. Childs and E. J. Forbes, *ibid.*, 2024 (1959).

(9) *Cf.* W. E. Noland and R. F. Lange, THIS JOURNAL, **81**, 1203 (1959), and ref. cited therein.

(10) K. T. Potts and J. E. Saxton, *J. Chem. Soc.*, 2641 (1954).

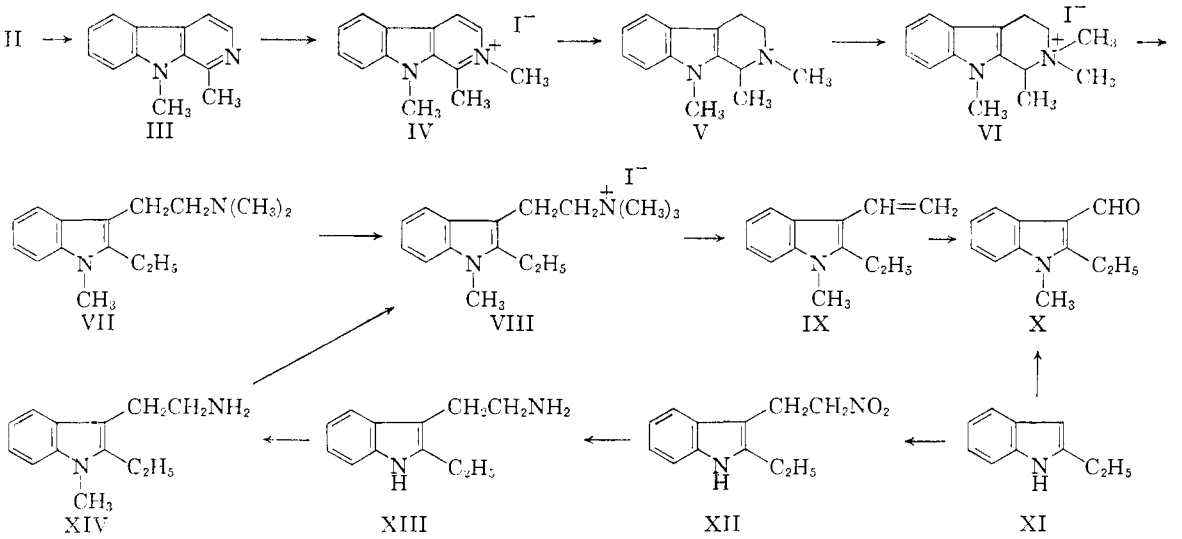


Fig. 1.—Degradation of the radioactive ajmaline and related reactions.

(see also Experimental). This vinylindole reacted rapidly with osmium tetroxide in ether to yield an osmate ester which was decomposed with alkaline sodium sulfite. The resultant diol was cleaved with periodate to yield formaldehyde, which was isolated as its dimedone derivative, and 2-ethyl-1-methyl-3-indolealdehyde (X). This aldehyde was identical with a specimen prepared by an unambiguous route from 2-ethylindole. 2-Ethylindole was formylated in the 3-position by the Vilsmeier method using the procedure of Smith[12] and then methylated on the 1-position with dimethyl sulfate in the presence of potassium hydroxide.[13]

The aldehyde X derived from the radioactive ajmaline was inactive, but the formaldehyde-dimedone derivative and all the other intermediates in this degradative scheme had essentially the same specific activity as the ajmaline (*cf.* Table I). This indicates that the ajmaline was labeled solely on the carbon atom represented by a heavy dot in formula II.

In future papers in this series we will report the results of the systematic degradation of the radioactive serpentine and reserpine derived from the tryptophan-2-C^{14} and our investigations on the origin of the intact or fragmented "E" ring of these alkaloids.

The 6-methoxytryptophan-3-C^{14} used in our preliminary feeding experiments was prepared from 6-methoxyindole *via* 6-methoxygramine using the method which Snyder and Smith[14] developed for tryptophan. This aminoacid has been previously prepared from 6-methoxy-3-indolealdehyde *via* the hydantoin[15] and by the condensation of 6-methoxygramine with ethyl acetylaminoöxaloacetate.[16]

(11) J. Szmuszkovicz, THIS JOURNAL, **82**, 1180 (1960).
(12) G. F. Smith, *J. Chem. Soc.*, 3842 (1954).
(13) H. Wieland, W. Konz and H. Mittasch, *Ann.*, **513**, 1 (1934).
(14) H. R. Snyder and C. W. Smith, THIS JOURNAL, **66**, 350 (1944).
(15) D. G. Harvey and W. Robson, *J. Chem. Soc.*, 97 (1938).
(16) R. G. Jones and E. C. Kornfeld, U. S. Patent 2,621,187 (*cf. Chem. Abs.*, **47**, 10557 (1953)).

Experimental[17]

Administration of DL-Tryptophan-2-C^{14} to *R. serpentina* and Isolation of the Alkaloids.—Two *R. serpentina* plants[18] (3 years old) were grown in soil, the top leaves being about 50 cm. below six 40 watt tubular fluorescent lights. The stems were threaded with a cotton darning thread by means of a needle[19] about 10 cm. above the surface of the soil. The ends of the thread were placed in beakers containing DL-tryptophan-2-C^{14} [20] (37.5 mg., 2.2×10^8 c.p.m.) dissolved in 0.1 *N* hydrochloric acid (10 ml.). The solution was all absorbed by the plants in 3 hr. More water was fed to the plants through this wick arrangement for 3 days. They were then watered normally and harvested 45 days after feeding the tracer.

The plants (wet wt. 140 g.) were mascerated in a Waring Blendor with chloroform (1000 ml.) and 15 *N* ammonia (10 ml.). The Blendor was washed out with additional chloroform (500 ml.). When, after standing several days, two layers had separated the mixture was filtered through cloth. The alkaline aqueous layer contained 19% of the activity which had been administered to the plants. The chloroform layer which contained 12% of the activity fed was evaporated to 130 ml. and extracted with *N* potassium dihydrogen phosphate solution (5×200 ml.) which dissolved the strong bases, ajmaline and serpentine. The residual chloroform layer was evaporated to 10 ml. and diluted with petroleum ether (100 ml.). This solution was extracted with a mixture of *N* sulfuric acid (300 ml.) and methanol (150 ml.). The filtered aqueous solution was made alkaline with ammonia and extracted with chloroform. The dried chloroform extract was evaporated to yield a residue (165 mg.) having a total activity of 7.6×10^6 c.p.m. This residue was dissolved in benzene (40 ml.), diluted with petroleum ether (10 ml.) and chromatographed on Woelm alumina[21] (activity III) (60 g. in a column, 2.5 cm. diameter). The column was eluted with benzene, 30 ml. fractions being collected. When 11 fractions had been collected the eluting solvent was

(17) All melting points are corrected. Microanalyses were determined by Mrs. Olga Hamerston and her assistants at the University of Minnesota. Radioactivity counts 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. The petroleum ether had b.p. 60–70°.

(18) The author is very grateful to Dr. J. E. Campion of the Riker Laboratories, Inc., Northridge, California, for procuring the plants from Columbo, Ceylon. The seedlings survived the trip by air express and flourished under greenhouse conditions in Minnesota.

(19) *Cf.* C. L. Comar, "Radioisotopes in Biology and Agriculture," McGraw-Hill Book Co., New York, N. Y., 1955, p. 151.

(20) Purchased from Tracerlab Inc., Waltham, Mass.

(21) Purchased from Alupharm Chemicals, New Orleans, La.

changed to a mixture of benzene and acetone (2:1 by volume). Evaporation of fractions 14 and 15 yielded crystalline reserpine (44 mg.) which after several crystallizations from acetone–methanol had a constant specific activity of 5.4×10^5 c.p.m./mM. The isolation procedure is essentially that of Dorfman, *et al.*[22]

The aqueous solution containing the ajmaline and serpentine was made basic with concentrated ammonia and extracted with chloroform. The dried chloroform extract was evaporated and the residue dissolved in benzene and chromatographed on alumina (activity III) which was eluted in turn with benzene, benzene–chloroform (1:1), chloroform and 10% methanol in chloroform. This last solvent eluted the ajmaline and serpentine together (282 mg., having an activity of 9.3×10^6 c.p.m.). This mixture was separated by the method of Schlittler and Schwarz.[23] The crude ajmaline (80 mg.) was purified by crystallization of its hydrochloride from ethanol–ether and ultimately had a constant specific activity of 2.5×10^6 c.p.m./mM. The serpentine was isolated as its nitrate (5.7 mg.) which after several crystallizations had a constant activity of 1.2×10^6 c.p.m./mM.

The purity and degree of separation of the alkaloids was checked during the isolation procedures by paper chromatography.[24]

Degradation of the Ajmaline-C^{14}.—The radioactive ajmaline isolated from the plant was diluted with inactive ajmaline[25] (597 mg.) and the degradation carried out on this material.

N-(ind)-Methylharman[6] (III).—Ajmaline (500 mg.) containing methanol of crystallization was intimately mixed in a mortar with soda-lime (7.5 g.) and then dried *in vacuo* at 100° for 24 hr. The mixture was placed in a conical flask and covered with a layer of glass wool. Air was flushed out with nitrogen and the flask then placed in a metal bath at 120°. The temperature was raised to 285° during 1 hr. and kept at this temperature for 12 hr. Some yellow material sublimed onto the glass wool. After cooling, the contents of the flask were comminuted in a Waring Blendor with dry ether (500 ml.). The filtered ether extract was evaporated to 20 ml. and treated with a ethanolic solution of hydrochloric acid. A pale yellow precipitate separated (165 mg.) which was dissolved in water and made alkaline with sodium hydroxide. The white precipitate produced was extracted with ether. The brown oil remaining after removal of the ether was chromatographed on alumina (activity III), eluting with benzene. Evaporation of the first fraction (100 ml.) yielded an oil which did not crystallize. The second fraction (100 ml.) yielded an oil which solidified on triturating with petroleum ether. Crystallization from benzene–petroleum ether yielded almost colorless prisms of N-(ind)-methyl harman (52 mg. 19%) identical with authentic material (mixed melting point, infrared spectrum).

N-(ind)-Methylharman-Nβ-methiodide (IV).—N-(ind)-Methylharman (50 mg.) was refluxed in methanol (2 ml.) with methyl iodide (0.5 ml.) for 3 hr., then allowed to cool overnight. A small amount of ether was added and the yellow precipitate of the methiodide filtered off (74 mg., 88%) m.p. 307–308° dec.

Anal. Calcd. for $C_{14}H_{15}N_2I$: C, 49.72; H, 4.47; N, 8.28. Found: C, 49.90; H, 4.66; N, 8.30.

N-(ind)-Methyl-1,2-dimethyl-1,2,3,4-tetrahydro-β-carboline-Nβ-methiodide (VI).—The active methiodide IV was diluted with inactive material to give a total weight of 199 mg. This was suspended in boiling absolute ethanol (20 ml.), sodium borohydride (100 mg.) added and the mixture refluxed for 5 min. when a colorless solution was obtained. The solution was allowed to cool for 2 hr., then the alcohol was removed *in vacuo*. The residue was suspended in 1% sodium hydroxide solution and the oil extracted with ether. The dried ether extract was evaporated and the residue refluxed in methanol (5 ml.) with methyl iodide (0.5 ml.) for 2 hr. The solution was then evaporated to small bulk and diluted with ether when the methiodide separated as fine, very pale yellow needles (165 mg., 79%), m.p. 246–247°.

Anal. Calcd. for $C_{15}H_{21}N_2I$: C, 50.56; H, 5.94; N, 7.86. Found: C, 50.70; H, 6.09; N, 7.72.

2-Ethyl-1-methyl-N,N-dimethyltryptamine (VII).—The methiodide VI (160 mg.) was suspended in liquid ammonia (20 ml.) and sodium (37 mg.) added, the ammonia being allowed to evaporate at room temperature. After 2 hr. more ammonia (20 ml.) and sodium (5 mg.) were added. When this ammonia had evaporated the orange residue was treated with water (20 ml.) and extracted with ether. The dried ether extract was evaporated and the residue distilled (190°(0.01)) to yield a very pale yellow oil (92 mg., 89%).

Anal. Calcd. for $C_{15}H_{22}N_2$: C, 78.21; H, 9.63; N, 12.16. Found: C, 78.11; H, 9.34; N, 11.87.

The **picrate** was obtained as orange prisms from 95% ethanol, m.p. 174–175°.

Anal. Calcd. for $C_{15}H_{22}N_2 \cdot C_6H_3N_3O_7$: C, 54.89; H, 5.48; N, 15.24. Found: C, 55.21; H, 5.44; N, 15.07.

2-Ethyl-1-methyl-N,N-dimethyltryptamine Methiodide (VIII).—The tertiary base VII (92 mg.) was refluxed with methanol (1 ml.) and methyl iodide (0.3 ml.) for 2 hr., then evaporated to small bulk and diluted with ether when colorless needles of the methiodide separated (136 mg., 91%), m.p. 216–217°. The analytical sample was crystallized from ethanol yielding needles, m.p. 220–221°.

Anal. Calcd. for $C_{16}H_{25}N_2I$: C, 51.47; H, 7.02; N, 7.51. Found: C, 50.99; H, 6.64; N, 7.46.

Hofmann Degradation of the Methiodide VIII and Oxidation of the Product.—The methiodide VIII (131 mg.) was dissolved in hot water (7 ml.), and moist silver hydroxide prepared from silver nitrate (170 mg.) was added and stirred for 10 min. The mixture was filtered and the filtrate evaporated to dryness *in vacuo* at 40°. The residue was distilled (175° (10^{-3} mm.)) to yield 2-ethyl-1-methyl-3-vinylindole (IX) as a colorless oil (61.3 mg., 94%). This oil became yellow on standing in air and no attempt was made to analyze this compound. The ultraviolet spectrum of the freshly distilled liquid in 95% ethanol had the following maxima and inflections: 233 mμ (ϵ 30,400), 265 mμ (ϵ, 20,000), 288 mμ (ϵ 12,000), inflections at 258, 294, 306 mμ.

Osmium tetroxide (85.2 mg.) in ether (5 ml.) was added to a rapidly stirred solution of the vinylindole (61.3 mg.) in ether (5 ml.) containing pyridine (0.1 ml.) cooled to −70°. More ether (5 ml.) was added to wash out the dropping funnel. A buff colored precipitate separated almost immediately. After stirring for 30 min. at −70° the mixture was allowed to warm up to room temperature, and after 2 hr. the osmate ester–pyridine complex was filtered off and washed with ether.

The complex was dried in air and then added to a solution of sodium sulfite (125 mg.) and potassium carbonate (40 mg.) in 50% ethanol (4 ml.) and shaken for 30 min. The brown solution was then extracted several times with ether. The yellow ether extract was evaporated *in vacuo* and the residue dissolved in a little water and treated with a solution of potassium periodate (100 mg.) in water (20 ml.). A crystalline precipitate of 2-ethyl-1-methyl-3-indolealdehyde (X) separated (32 mg., 52% yield from the vinylindole), m.p. 97–98°. The sample which was assayed for radioactivity was obtained by sublimation of this product *in vacuo* (160° (10^{-3} mm.)). It was identical (infrared spectrum, mixed m.p.) with a specimen of the aldehyde prepared by an unambiguous route described later.

The filtrate obtained after removal of the indolealdehyde was distilled and the aqueous distillate added to a solution of dimedone (100 mg.) in water (50 ml.). After standing overnight the formaldehyde-dimedone derivative separated out as fine colorless prismatic needles (45 mg., 46%), m.p. 194°, not depressed on admixture with an authentic specimen. It was crystallized from aqueous methanol prior to radioactive assay.

The activity of the ajmaline and its degradation products are recorded in Table I.

2-Ethyl-3-indole Aldehyde.—2-Ethylindole[26] (7.0 g.) was dissolved in dimethylformamide (30 ml.) and cooled to −30°. Phosphorus oxychloride (7.6 ml.) was added to the stirred solution which was allowed to warm up to room

(22) L. Dorfman, A. Furlenmeier, C. F. Heubner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. Andre, *Helv. Chim. Acta*, **37**, 59 (1954).

(23) E. Schlittler and H. Schwarz, *ibid.*, **33**, 1463 (1950).

(24) *Cf.* F. Kaiser and A. Popelak, *Ber.*, **92**, 278 (1959).

(25) The author thanks Dr. J. E. Campion of the Riker Laboratories, California and Dr. W. I. Taylor of Ciba, Inc., New Jersey for the generous supplies of the *Rauwolfia* alkaloids.

(26) A. Verley and J. Beduwé, *Bull. soc. chim. France*, [4], **37**, 190 (1925).

TABLE I

ACTIVITY OF AJMALINE AND ITS DEGRADATION PRODUCTS C.P.M./mM (CARRIER FREE MATERIAL)

Ajmaline (II)	2.5×10^6
N-(ind)-Methylharman (III)	2.3×10^6
N-(ind)-Methylharman-Nβ-methiodide (IV)	2.3×10^6
N-(ind)-Methyl-1,2-dimethyl-1,2,3,4-tetrahydro-β-carboline-Nβ-methiodide (VI)	2.2×10^6
2-Ethyl-1-methyl-N,N-dimethyltryptamine methiodide (VIII)	2.0×10^6
2-Ethyl-1-methyl-3-indole aldehyde (X)	0
Formaldehyde dimedone derivative	2.4×10^6

temperature overnight. The mixture was then added to crushed ice, neutralized with sodium hydroxide and boiled for 1–2 min. The solution was cooled and the crude aldehyde filtered off and dried. Crystallization from ethanol (20 ml.) yielded fine colorless prismatic needles of 2-ethyl-3-indole aldehyde (7.2 g.), m.p. 170–171°.

Anal. Calcd. for $C_{11}H_{11}NO$: C, 76.27; H, 6.40; N, 8.09. Found: C, 76.53; H, 6.48; N, 8.17.

2-Ethyl-1-methyl-3-indole Aldehyde. (X).—2-Ethyl-3-indole aldehyde (5.7 g.) was suspended in a solution of potassium hydroxide (24 g.) in 100 ml. of water at 80°. Dimethylsulfate (9 ml.) was added to this hot solution and shaken. Heat was evolved and a homogeneous mixture was obtained. After a few minutes the product separated out as an oil which solidified on cooling (6.0 g.). Several crystallizations from benzene–petroleum ether yielded colorless needles of 2-ethyl-1-methyl-3-indole aldehyde, m.p. 100–101°. The infrared spectrum (KBr pellet) showed a C=O absorption at 1640 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{13}NO$: C, 76.97; H, 7.00; N, 7.48. Found: C, 76.89; H, 7.40; N, 7.63.

2-Ethyl-3-(2-nitroethyl)-indole (XII).—2-Ethylindole (1.45 g.) dissolved in benzene (10 ml.) was added rapidly with stirring to nitroethylene[27] (0.8 ml.) dissolved in benzene (10 ml.) cooled to 5°. The mixture became dark brown after a few minutes and was stirred at room temperature for 12 hr. The solution was then evaporated to dryness *in vacuo* and the residual brown oil distilled (200° (0.01 mm.)) to yield an almost colorless oil which crystallized on cooling (1.27 g.), m.p. 65–71°. Recrystallization from benzene–petroleum ether yielded fine colorless needles of 2-ethyl-3-(2-nitroethyl)-indole, m.p. 77–78°. The infrared spectrum showed an NH absorption at 3400 cm.$^{-1}$ and an NO_2 absorption at 1550 cm.$^{-1}$ (KBr pellet).

Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.03; H, 6.47; N, 12.84. Found: C, 66.49; H, 6.51; N, 13.08.

2-Ethyltryptamine (XIII).—The nitroethyl derivative XVIII (1.47 g.) was dissolved in 95% ethanol (40 ml.) and hydrogenated at a pressure of 2 atm. in the presence of platinum oxide (0.3 g.) for 6 hr. The platinum was then filtered off and the filtrate evaporated to dryness. The residue was dissolved in benzene and extracted with 2 *N* sulfuric acid (100 ml.). The aqueous extract was made basic with sodium hydroxide and extracted with ether. The dried ether extract was evaporated and the residue distilled (160° (0.05 mm.)) to yield 2-ethyltryptamine as a pale yellow viscous oil (0.61 g.).

The phthalimide was prepared by refluxing the amine with phthalic anhydride in acetic acid for 1 hr. Crystallization of the product from aqueous methanol yielded pale yellow needles, m.p. 161–162°.

Anal. Calcd. for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.45; H, 5.87; N, 8.71.

The picrate was obtained as orange needles from ethanol, m.p. 216–217°.

Anal. Calcd. for $C_{12}H_{16}N_2 \cdot C_6H_3N_3O_7$: C, 51.80; H, 4.59; N, 16.78. Found: C, 52.10; H, 4.49; N, 16.77.

2-Ethyl-1-methyltryptamine (XIV).—Sodium (85 mg.) was dissolved in liquid ammonia (40 ml.), a crystal of ferric nitrate being added to catalyze reaction with the solvent. 2-Ethyltryptamine (550 mg.) in liquid ammonia (30 ml.) was added and then a few minutes later methyl iodide (0.2 ml.). The solution became turbid and lost its bluish violet fluorescence. The ammonia was allowed to evaporate and water added to the residue. The oil which separated was extracted with ether and distillation of the dried ether extract yielded a pale yellow oil (510 mg.), b.p. 150° (0.05 mm.).

The phthalimide was obtained as yellow plates from ethanol, m.p. 141–142°.

Anal. Calcd. for $C_{21}H_{20}N_2O_2$: C, 75.88; H, 6.07; N, 8.43. Found: C, 75.96; H, 6.04; N, 8.43.

2-Ethyl-1-methyl-N,N-dimethyltryptamine Methiodide (VIII).—2-Ethyl-1-methyltryptamine (200 mg.) was refluxed in methanol (5 ml.) with methyl iodide (0.5 ml.) and sodium bicarbonate (180 mg.) for 3 hr. The mixture was then filtered and the filtrate evaporated to about 2 ml. and a small amount of ether added. The methiodide separated out as colorless needles (258 mg.), m.p. 220–221°.

This material was identical (infrared spectrum, mixed m.p.) with the compound obtained from the Emde reduction product of the methiodide XII.

N-(ind)-Methylharman (III).—This was prepared by the methylation of harman in liquid ammonia[6] and from N-(ind)-methyltryptophan[28] by the following procedure. N-(ind)-Methyltryptophan (1.5 g.) was dissolved in *N* sulfuric acid (7.5 ml.) and then 10% aqueous acetaldehyde (33 ml.) added and the mixture slowly raised to 100° during 2 hr. The solution was then boiled for 10 min. in an open beaker, then cooled and made just alkaline with ammonia. This solution was then added to boiling water (500 ml.), then a solution of potassium dichromate (9 g.) and acetic acid (18 ml.) in water (90 ml.) was added and the mixture boiled for 1 min. and then allowed to cool for 15 min. Sulfur dioxide was then passed into the solution until it became green. The cooled mixture was then made strongly alkaline and extracted with ether. Evaporation of the dried ether extract yielded N-(ind)-methylharman (1.06 g., 63%). Crystallization from benzene–petroleum ether yielded colorless prisms, m.p. 101.5–102.5° (lit.,[6] 102°).

Preliminary Feeding Experiments. (a) **6-Methoxytryptophan-3-C^{14}.**—DL-6-Methoxytryptophan-3-C^{14} (100 mg., 4.2×10^7 c.p.m.), was added to the nutrient solution[29] in which the roots of four *R. serpentina* plants (one year old) were growing. The tracer was absorbed slowly by the plants and at the time of harvesting (40 days after the initial feeding) the nutrient solution contained 25% of the initial activity fed. The reserpine isolated from the plants (wet wt. 100 g.) was inactive.

(b) **Tryptophan-3-C^{14}.**—DL-Tryptophan-3-C^{14}[30] (628 mg., 6.7×10^7 c.p.m.) was added to the nutrient solution in which two *R. serpentina* plants (2 years old) were growing. After 20 days 2.5% of the initial tracer fed remained in the nutrient solution. The plants were harvested at this time (wet wt. 56 g.). A very small amount of activity was detected in the reserpine, ajmaline and serpentine isolated from these plants; however this was not sufficient to enable us to carry out extensive degradation of the alkaloids.

(c) **Tyrosine-2-C^{14}.**—DL-Tyrosine-2-C^{14}[20] (50 mg., 2.7×10^8 c.p.m.) was added to the nutrient solution in which the roots of one *R. serpentina* plant (2 years old) were growing. The tracer disappeared relatively quickly from the nutrient solution. After 2 days only 11% of the initial activity remained. The plant was harvested 21 days after the initial feeding. The reserpine, serpentine and ajmaline isolated from the plant (wet wt. 58 g.) were inactive.

6-Methoxytryptophan-3-C^{14}.—6-Methoxyindole[31] (0.90 g., 6.1 m*M*) was added to a solution obtained by the addition of formaldehyde -C^{14} (0.185 g. in 15 ml. of water, 6.1 m*M*, 0.5 mc.) to an ice cold mixture of acetic acid (4.5 ml.) and 50% dimethylamine (1.0 ml.). The mixture was shaken for 4 hr. and then allowed to stand overnight at room temperature. The solution was then filtered and the filtrate made alkaline with sodium hydroxide (6.0 g.). 6-Methoxygramine was filtered off and dried in air (1.03 g.). An inactive sample crystallized from benzene–petroleum ether afforded colorless prisms, m.p. 88–89°.

(27) The author thanks Dr. W. E. Noland and Mr. R. W. Campbell of the University of Minnesota for the sample of nitroethylene.

(28) E. Leete, *J. Org. Chem.*, **23**, 631 (1958).

(29) Of the same composition as used in our experiments with tobacco: E. Leete, THIS JOURNAL, **78**, 3520 (1956).

(30) K. Bowden and L. Marion, *Can. J. Chem.*, **29**, 1037 (1951).

(31) W. O. Kermack, W. H. Perkin and R. Robinson, *J. Chem. Soc.*, **119**, 1602 (1921).

Anal. Calcd. for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.90. Found: C, 70.23; H, 7.71.

Ethylacetylaminomalonate (1.03 g.) was added to a solution of sodium (0.11 g.) in dry ethanol (11 ml.) with stirring at room temperature. 6-Methoxygramine (1.03 g.) was then added to the mixture which was then cooled to 0° and dimethyl sulfate (0.94 ml.) added. After stirring overnight at room temperature the mixture was added to ice and the product crystallized out (1.56 g.). The analytical sample was crystallized from ethanol to yield colorless prisms of ethyl-α-acetamido-α-carbethoxy-β-(3-(6-methoxyindole))-propionate, m.p. 147–148°.

Anal. Calcd. for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64. Found: C, 59.68; H, 6.38.

This ester (1.56 g.) was refluxed with 10% sodium hydroxide solution (10 ml.) for 1 hr., then cooled and acidified with concentrated hydrochloric acid and the malonic acid derivative filtered off and washed with cold water. The residue was refluxed with water (40 ml.) for 6 hr., then sodium hydroxide (3.0 g.) added and the mixture refluxed overnight. The solution was then made acidic with 2*N* sulfuric acid and then basic to phenolphthalein with barium hydroxide solution. The mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in boiling acetic acid (40 ml.), filtered and benzene (40 ml.) added when 6-methoxytryptophan acetate (0.76 g., 42% yield from the formaldehyde-C^{14}) crystallized out as colorless plates, m.p. 277–278°.

Anal. Calcd. for $C_{12}H_{14}N_2O_3 \cdot C_2H_4O_2$: C, 57.13; H, 6.17. Found: C, 57.37; H, 6.22.

In an inactive run the α-acetamido-α-carboxy-β-(3-(6-methoxyindole))-propionic acid was isolated and crystallized from aqueous ethanol to afford colorless prisms, m.p. 175–176° dec.

Anal. Calcd. for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04. Found: C, 56.56; H, 5.00.

This malonic acid derivative was decarboxylated by heating in a nitrogen atmosphere at 160–180° for 30 min. The residue was crystallized from ethanol to yield N-acetyl-6-methoxy-tryptophan as colorless rhombic prisms, m.p. 209–210°.

Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.48. Found: C, 61.13; H, 5.86.

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Syntheses of Bicyclo[3.3.0]octane Derivatives

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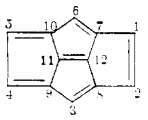
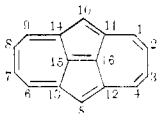
RECEIVED MAY 10, 1960

From calculations using simple molecular orbital theory, the hypothetical hydrocarbons I and II are predicted to be stable aromatic compounds. In an attempt to test these predictions the Diels–Alder reaction between bicyclo[2.2.1]heptadiene and cyclopentadiene has been utilized to prepare a series of 2,4,6,8-tetrasubstituted bicyclo[3.3.0]octane derivatives. Although their further conversion to I and II was not successful, a correlation between these derivatives and the Schroeter and Vossen "red salt" was made. Attempts to convert 2,4,6,8-tetraaminobicyclo[3.3.3]octane to pentalene are described.

Although the generalization from molecular orbital theory known as the "Hückel rule" is not applicable to polycyclic molecules containing fused rings,[3] previous studies would suggest that the detailed molecular orbital calculations following the standard Hückel method[4] do provide useful predictions concerning the properties of such polycyclic molecules.[5,6] To extend these studies it would be of particular interest to test the predictions of simple molecular orbital theory for polycyclic molecules having no benzenoid rings. In this communication the molecular orbital calculations for hydrocarbons I and II are presented and experiments directed toward their synthesis are described.



I



II

The calculations followed the standard Hückel method[4,7] with all coulomb integrals considered equal, as were all resonance integrals between adjacent carbons, and overlap was neglected. An I.B.M. 650 computer was employed for the calculations using a standard matrix-diagonalization program. The charge densities, bond orders and free valences for hydrocarbons I and II are summarized in Tables I and II. The delocalization (resonance) energy for I is 4.19 β whereas that of II is 6.03 β.

There are several points of interest with regard to these calculations, probably the foremost being the prediction of a high degree of thermodynamic stability for both I and II. This is in accord with the qualitative predictions deduced from other theoretical approaches. Thus, Platt used the box model with the free-electron network theory to arrive at the prediction that I (pyranene) would be a stable aromatic compound.[8] The predictions for I and II based on the symmetry requirements of Craig[9] are ambiguous. Although angle strain and bond compressions, which have been neglected in our calculations, would undoubtedly destabilize I, and to a lesser extent II, the predicted delocalization energies per π-electron are surprisingly high for I and II (0.35 and 0.38 β, respectively) and are in the same range as naphthalene (0.38 β) or anthracene (0.38 β). Furthermore, it is clear that the central bonds are involved in the resonance stabilization, since isolation of C_{11}–C_{12} in I leaves a system with a delocalization energy of 2.94 β (0.29 β per π-electron) and isolation of C_{15}–C_{16} in II leaves a sys-

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