739. *On the Origin of the* C-1 *Fragment in Indole Alkaloids*

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Evidence is presented that the C-1 (C-21) unit in ajmaline is not derived from *N*-methyltryptophan, *N*-methyltryptamine, or methionine. Experimental support has been secured for the view that deoxyajmaline is the intimate precursor of ajmaline.

The origin of C-21 in ajmaline (I) has been the subject of recent, and conflicting, biosynthetic studies. Edwards and Leete [1] reported that ajmaline derived from sodium [^{14}C]-formate contained 12% of its activity at this position. More recently Battersby and his colleagues,[2] in a similar experiment, found negligible incorporation of formate into C-21 although 25% of the activity of the alkaloid was located in the *N*-methyl group, a typical C-1 unit.

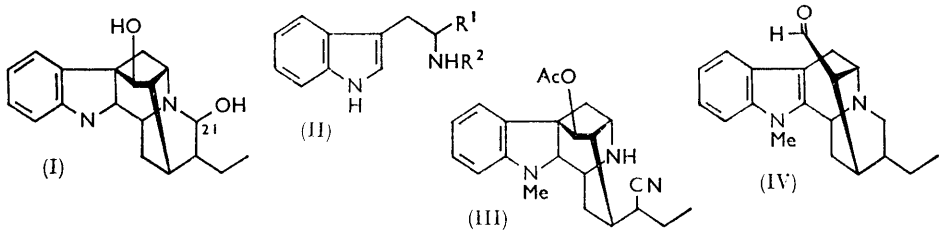
It was considered possible [3] that the C-21 bridge in ajmaline, and in other indole alkaloids, might arise by oxidative cyclisation of an *N*-methyl group. This process would be analogous to the formation of the C-1 bridge in the berberine alkaloids.[4] To test this idea, *N*-methyltryptophan (II; $R^1 = CO_2H$, $R^2 = Me$) and *N*-methyltryptamine (II; $R^1 = H$, $R^2 = Me$), both labelled with ^{14}C in the *N*-methyl groups, were fed separately to excised branches of *Rauwolfia verticillata* (*chinensis*). In neither experiment was significant incorporation into ajmaline observed, although a control feeding with [3-^{14}C]-tryptophan, a known [5] precursor of ajmaline, gave radioactive alkaloid (0·28% incorporation). The basic extract from the *N*-methyltryptophan feeding was diluted with inactive

[1] P. N. Edwards and E. Leete, *Chem. and Ind.*, 1961, 1666.
[2] A. R. Battersby, R. Binks, W. Lawrie, G. V. Parry, and B. R. Webster, *Proc. Chem. Soc.*, 1964, 369.
[3] D. H. R. Barton, Hugo Muller Lecture, *Proc. Chem. Soc.*, 1963, 293.
[4] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *Proc. Chem. Soc.*, 1963, 267; A. R. Battersby, R. J. Francis, M. Hirst, and J. Staunton, *ibid.*, p. 268.
[5] E. Leete, *J. Amer. Chem. Soc.*, 1960, **82**, 6338.

N-methyltryptamin, and this base re-isolated. After purification the *N*-methyltryptamine was found to contain 0·54% of the original activity fed. This biological decarboxylation provides further reason for believing that *N*-methyltryptophan is not a precursor of ajmaline.

The work of Battersby and his colleagues [2] suggests that C-21 in ajmaline is not derived, in a simple manner, from a biological C-1 unit. To obtain further evidence on this point (±)-[*methyl*-^{3}H]methionine was wick-fed (see Experimental section) to a mature *Rauwolfia serpentina* plant. *R. serpentina*, though difficult to grow, is a better source of ajmaline than *R. verticillata*. Incorporation (0·029%) into ajmaline was observed, and the specific activity of the alkaloid did not drop significantly after reduction to dihydroajmaline. Herzig–Meyer demethylation of this dihydro-compound gave methyl iodide containing 97% of the original activity. A more sensitive proof that no tritium was attached to C-21 was obtained in the following way. Treatment of the derived radioactive ajmaline oxime with hydrogen chloride in acetic acid containing acetic anhydride [6] gave the nitrile acetate (III). This derivative had the same specific activity as ajmaline. Providing that one of the hydrogen atoms in the *S*-methyl group of methionine is retained during biosynthesis, this result shows that a C-1 unit is not a precursor for C-21 of ajmaline. This conclusion would be invalidated, for example, if ajmaline were derived biologically from the related bridged lactam. However, we have preliminary evidence that deoxyajmaline [as (I) but lacking hydroxyl at C-21] is a precursor of the alkaloid. Deoxyajmaline, labelled with tritium in the aromatic ring by exchange in dilute [^{3}H]sulphuric acid, was fed to an excised branch of *R. verticillata*. The incorporation (0·048%) was greater than that (0·013%) for tryptophan in a parallel experiment. In a similar way the conversion of triturated deoxyajmalal-A (IV), prepared from the labelled deoxyajmaline, into ajmaline in *R. verticillata* was observed. In this case, however, the incorporation was very low (0·003%). Clearly, further work with specifically labelled precursors could profitably be done to define the late stages of ajmaline biosynthesis. For example, *N*-nordeoxyajmalal-A might be a more likely precursor than the *N*-methyl compound (IV).

We conclude that the oxidative cyclisation of an *N*-methyl is not involved in the biosynthesis of ajmaline, a typical indole alkaloid, and that this process is probably not of importance for indole alkaloids in general.



A convenient synthesis of tryptamine by controlled decarboxylation of tryptophan is described in the Experimental section.

Experimental

All n.m.r. spectra were run on a Varian A-60 spectrometer on permanent loan from the Wellcome Trust. Melting points were measured on a Kofler hot-stage apparatus.

Counting Methods.—The activities of ^{14}C-compounds were measured on thin films in a windowless counter.[7] Tritium activities were determined with a scintillation counter [8] and corrections applied, when necessary, for quenching by the labelled compound. Appreciable quenching

[6] F. A. L. Anet, D. Chakravarti, Sir Robert Robinson, and E. Schlittler, *J.*, 1954, 1242.
[7] See D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J.*, 1963, 4545.
[8] See D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, *J.*, 1965, 2423.

was observed with methyltriethylammonium iodide from the Herzig–Meyer demethylation of ajmaline (see below) and was determined separately using the inactive salt and standard [1,2-$^3H_2$]hexadecane.

Feeding of Precursors and Isolation of Ajmaline.—The freshly cut ends of excised branches of *Rauwolfia verticillata* were placed in aqueous solutions (*ca.* pH 6) of precursors contained in narrow tubes. The mouths of the tubes were sealed around the branch and a stream of warm air directed upon the leaves. Rapid uptake of the solutions was observed. Distilled water was added to the tubes from time to time to prevent withering of the plant. The plant material was worked up after 4—7 days. A young flowering plant of *Rauwolfia serpentina* was fed through a cotton wick in the usual way [5] and worked up after 11 days.

Plant material was extracted by established methods [5] and the ajmaline-rich, strongly basic fraction (typically 20 mg. from a small branch) chromatographed on alumina (grade III). Elution with chloroform was controlled by thin-layer chromatography on silica gel G (Merck) plates developed in methanol. Ajmaline (R_F *ca.* 0·4) was detected with iodine vapour. The ajmaline-containing fractions were combined, evaporated, and diluted with pure inactive alkaloid. The ajmaline was recrystallised (as the methanolate) from methanol to constant activity. Conversion into the hydrochloride provided a further test of radiochemical purity.

(±)-[methyl-^{14}C]-N-*Methyltryptophan.*—This precursor was prepared from (±)-tryptophan and [^{14}C]methyl iodide.[9] Its purity was confirmed by paper chromatography.[10]

[methyl-^{14}C]-N-*Methyltryptamine* (with Dr. E. J. Herbert).—3-Indolylglyoxalyl chloride (17·2 mg.) and [^{14}C]methylamine hydrochloride (2·6 mg.) in dry dimethylformamide (0·7 ml.) were treated with excess (*ca.* 0·1 ml.) of triethylamine at room temperature overnight in a sealed ampoule. The mixture was diluted with 1N-sodium hydrogen carbonate (2 ml.) and extracted with chloroform (4 × 1 ml.). The extract was washed with 1N-hydrochloric acid (2 × 1 ml.) and with water (1 ml.), dried (Na_2SO_4), and evaporated to give the desired amide (6·5 mg.). A similar experiment with inactive materials gave N-*methyl*-3-*indolylglyoxamide*, m. p. 225—225·5° (from propan-2-ol) (Found: C, 65·3; H, 5·0; N, 13·8. $C_{11}H_{10}N_2O_2$ requires C, 65·3; H, 5·0; N, 13·9%).

This amide (5·8 mg.) in dry dioxan (1·5 ml.) was added slowly during 30 min. to a refluxing suspension of lithium aluminium hydride (*ca.* 100 mg.) and aluminium chloride (30 mg.) in dioxan (5 ml.). After 5 hr. refluxing the mixture was cooled and poured into a saturated solution of sodium sulphate (15 ml.). The product was extracted with ether (5 × 10 ml.). The ether layer was washed with water and then extracted with 2N-hydrochloric acid (3 × 5 ml.). The acidic solution was basified with ammonium (*d* 0·880) and extracted with chloroform (4 × ml.); this extract was dried (Na_2SO_4) and evaporated to give oily *N*-methyltryptamine (3·0 mg.). The radioactive material was assayed by dilution with inactive *N*-methyltryptamine and crystallisation of the hydrochloride (40% radiochemical yield from the amide). Reduction of inactive amide in these conditions gave *N*-methyltryptamine, m. p. 87—88° [from light petroleum (b. p. 80—100°)] (lit.,[11] 86—87°) (Found: C, 75·9; H, 8·1; N, 15·5. Calc. for $C_{11}H_{14}N_2$: C, 75·8; H, 8·1; N, 16·1%). The corresponding hydrochloride crystallised from ethanol–ether as flakes, m. p. 179—179·5° (lit.,[12] 180°) (Found: C, 62·5; H, 6·9; N, 14·3. Calc. for $C_{11}H_{15}ClN_2$: C, 62·7; H, 7·2; N, 13·3%).

(±)-[methyl-^{3}H]*Methionine.*—This precursor, prepared [13] from (±)-homocystine and [^{3}H]methyl iodide, was counted as its methyl ester hydrochloride. The latter was conveniently soluble in dimethylformamide.

Tritiation of Tryptophan.—(±)-Tryptophan (30 mg.) was heated at 100° for 4 hr. in [$^3H_2$] water (0·17 ml.; 3·6 mc per mmole) containing oleum (20% SO_3 in H_2SO_4; 0·03 ml.) in a sealed tube. The cooled solution was adjusted to pH 6 with 2N-sodium hydroxide and set aside to permit slow crystallisation of the amino-acid. The crystals were centrifuged off, washed with water (1 ml.), and recrystallised from aqueous ethanol to give (±)-[^{3}H]tryptophan (27 mg.), m. p. 281—285°. The activity of the product was determined after conversion into the

[9] H. Peter, M. Brugger, J. Schreiber, and E. Eschenmoser, *Helv. Chim. Acta*, 1963, **46**, 577.
[10] R. J. Blocke, E. L. Durrum, and G. Zweig, " Paper Chromatography and Paper Electrophoresis," Academic Press, New York, 1958, p. 319.
[11] L. A. Cohen, J. W. Daly, H. Kny, and B. Witkop, *J. Amer. Chem. Soc.*, 1960, **82**, 2184; T. Hoshino and K. Shinodaira, *Annalen*, 1935, **520**, 19.
[12] R. H. F. Manske, *Canad. J. Res.*, 1931, **5**, 592.
[13] W. Patterson and V. du Vigneaud, *J. Biol. Chem.*, 1935, **123**, 327.

N-acetyl derivative.[14] A parallel experiment in D_2O showed (n.m.r. spectrum) that *ca.* 80% of the aromatic hydrogens had exchanged; slight changes in the bands corresponding to the side-chain were also noted. A control run with ordinary water established that the crystalline product from these reactions was in fact (±)-tryptophan.

Tritiation of Deoxyajmaline.—Deoxyajmaline (51 mg.), prepared by pyrolysis of dihydroajmaline hydrobromide,[15] was heated at 100° for 70 hr. in [$^3H_2$]water (0·57 ml.) containing oleum (20% SO_3 in H_2SO_4; 0·10 ml.) as described above. The reaction product was made alkaline with 2N-sodium hydroxide and the precipitated alkaloid (47 mg.) was collected, washed with water, and dried *in vacuo*. Crystallisation of a portion (20 mg.) of this material from methanol gave plates (12 mg.), m. p. 293—295°. An experiment in D_2O (as above) indicated *ca.* 60% exchange of aromatic hydrogens under these conditions and the corresponding control run with ordinary water gave a good recovery of deoxyajmaline. Labelled deoxyajmalal-A was prepared from [^{3}H]deoxyajmaline by oxidation with lead tetra-acetate.[16]

O-*Acetylanhydroajmaline Oxime* (III).—Ajmaline oxime hydrochloride (1·00 g.), suspended in a mixture of acetic acid (7 ml.) and acetic anhydride (3 ml.), was treated with dry hydrogen chloride until a clear solution was obtained.[16] The crystals which separated after 2 hr. at 5° were collected, washed with ether, and dried *in vacuo* at 100° overnight. *O*-Acetylanhydroajmaline oxime dihydrochloride acetic acid solvate was obtained as rods (1·11 g.), m. p. 196—197° (lit.,[16] 202—205° after sintering at 198—200°) (Found: C, 57·8; H, 6·9; Cl, 13·9; N, 8·4; O, 12·9. Calc. for $C_{24}H_{33}Cl_2N_3O_4$: C, 57·8; H, 6·6; Cl, 14·3; N, 8·4; O, 12·9%). The infrared spectrum (Nujol) showed bands at 1750 (OAc) and 1710 cm.$^{-1}$ (HOAc). The nitrile band was obscured by broad absorption in the 2300 cm.$^{-1}$ region. Decomposition of the salt with sodium hydrogen carbonate and extraction into chloroform gave the free base showing infrared bands ($CHCl_3$) at 2280 (CN) and 1735 cm.$^{-1}$ (OAc). The salt was also prepared from anhydroajmaline oxime under the conditions used for ajmaline oxime. Hydrolysis of the salt with aqueous sodium hydroxide gave [16] anhydroajmaline oxime.

Feeding Experiments with R. Verticillata.—(±)-*N*-Methyltryptophan (1·5 mg.; 0·002 mc) labelled in the *N*-methyl group with ^{14}C gave (as above) a strongly basic fraction containing 2·4% of the activity fed. Chromatography and dilution gave inactive ajmaline. Dilution of the remaining strong bases with inactive *N*-methyltryptamine and purification *via* the hydrochloride showed an incorporation of 0·54% into this amine. A feeding experiment with the correspondingly labelled *N*-methyltryptamine (3 mg.; 0·023 mc) also gave inactive ajmaline. The labelled precursor was recovered (by dilution) in 26% yield and acccounted for essentially all the activity in the strongly basic fraction. A control experiment with (±)-[3-^{14}C]tryptophan performed at the same time gave radioactive ajmaline (0·28% incorporation).

In a later season a variety of tritiated precursors, prepared as described above, was fed to give the following incorporations into ajmaline: deoxyajmaline (5·5 mg.; 0·051 mc), 0·048%; deoxyajmalal-A (5·5 mg.; 0·051 mc), 0·003%; (±)-tryptophan (5 mg.; 0·11 mc), 0·013%; (±)-methionine (20 mg.; 0·214 mc), 0·012%. In the last experiment a larger branch (wet wt., 37 g.), giving 48 mg. of strong bases, was used to metabolise the increased quantity of precursor.

Feeding Experiment with R. Serpentina.—A wick-feeding experiment (see above) with (±)-[*methyl*-^{3}H]methionine (40 mg.; 0·43 mc) gave radioactive ajmaline (0·029% incorporation). Reduction with sodium borohydride gave dihydroajmaline (98% retention of the radioactivity). Demethylation of this derivative with hydrogen iodide and determination of the *N*-methyl group as methyltriethylammonium iodide revealed 97% of the tritium in this position. An error of 5% in this value is customary. The ajmaline was converted into the corresponding anhydroajmaline oxime salt (as above) having the same (100%) molar activity.

A Convenient Synthesis of Tryptamine.—DL-Tryptophan (1·00 g.) and diphenyl ether (50 ml.) were heated at reflux for 1 hr. in an atmosphere of nitrogen. The mixture was cooled and extracted with 2N-aqueous hydrochloric acid (3 × 40 ml.). This extract was washed with ether, basified (6N-aqueous sodium hydroxide), and extracted with ether (5 × 50 ml.). This extract was washed with water and brine, dried (sodium sulphate), and the solvent removed *in vacuo*, leaving a residue which crystallised from benzene giving pale yellow prisms (530 mg.), m. p.

[14] V. du Vigneaud and R. R. Scalock, *J. Biol. Chem.*, 1932, **96**, 511.
[15] Sir Robert Robinson, *Chem. and Ind.*, 1955, 285.
[16] M. F. Bartlett, R. Sklar, W. I. Taylor, E. Schlittler, R. L. S. Amai, P. Beak, W. V. Bringi, and E. Wenkert, *J. Amer. Chem. Soc.*, 1962, **84**, 622.

113—114°. Sublimation afforded a colourless crystalline solid (450 mg., 57%), m. p. 114—115°, which was identified with tryptamine by mixed m. p. and comparison of infrared spectra with an authentic specimen of the base.

The use of freshly distilled tetralin as the solvent for the decarboxylation led to a yield of only 36%. With commercial tetralin the yield was reduced to 20%. No tryptamine was isolated from experiments which employed diphenylamine or dimethyl sulphoxide in place of diphenyl ether.

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