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SHORT COMMUNICATION

BIOSYNTHESIS OF PALMATINE IN *FAGARA COCO*

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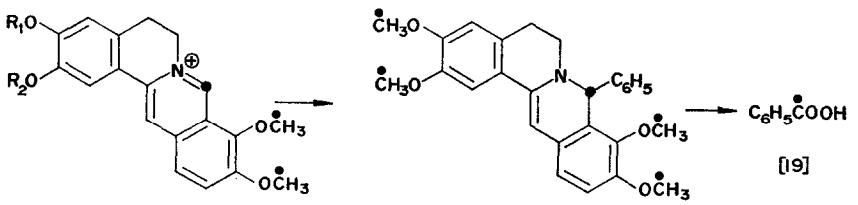
Abstract—The administration of sodium formate-^{14}C to *Fagara coco* yielded, among other alkaloids, radioactive palmatine (I). Degradation of I clearly showed that almost 20% of the tracer was located on the berberine bridge carbon.

PALMATINE (I) belongs to the protoberberine alkaloids. The biosynthesis of this type of alkaloids has been extensively studied by different research groups; berberine (II) and stylopine were the typical alkaloids used in those studies. Different compounds ranging from sodium formate and methionine[1] to more sophisticated labelled products[2-4] have been tested as precursors. All these investigations have shown that the so-called berberine bridge carbon[5] was derived by oxidative cyclization of the *N*-methyl group of a benzylisoquinoline alkaloid acting as precursor. (+) Reticuline is thought to be the intermediate in the reaction.[3]



I: $R_1=R_2=CH_3$ [100]

II: R_1, $R_2=CH_2$

III [88]

SCHEME. FIGURES IN BRACKETS REPRESENT RELATIVE SPECIFIC AVITIVITY.

Continuing with our work on the biosynthesis of alkaloids in *Fagara coco*[6] we now report that sodium formate-^{14}C acts as a precursor of the protoberberine alkaloid palmatine, and that degradation of the latter indicates, as expected, the formate origin of the berberine bridge carbon atom.

Sodium formate-^{14}C was fed in December 1968 into the stems of one intact *F. coco* plant through cotton wicks. After 12 days the plant was harvested, and radioactive palmatine

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(I) (specific incorporation: 0·0012%) and berberine (II) were isolated as described later. The former product was converted into 8-phenyldihydropalmatine (III) identified by UV and NMR spectroscopy;[7] the NMR spectrum of III in DMSO-d_6 showed, among other signals, two characteristic singlets at δ 5·73 and 6·03 attributable to H-8 and H-13 respectively; addition of one drop of DCl produced a paramagnetic shift of both signals indicating the deshielding of both protons by quaternization of the nitrogen atom.

Oxidation of III gave benzoic acid containing, within experimental error, 20% of the activity of the phenyldihydro derivative (see Scheme).

This result clearly indicates that, as in the case of berberine, C-8 of palmatine is directly derived from formate probably by the biological cyclization of an *N*-methyl group. With our radioactive berberine (II) we have confirmed the results found by Gupta and Spenser.[1]

As happens in other plants, (+) reticuline could be the intermediate in the biosynthesis of palmatine in *F. coco*; the fact that tembetarine, (+) *N*-methylreticuline, is found in *Fagara* species lacking protoberberine alkaloids whereas it is absent in *F. coco* which has this type of alkaloid,[8] could indicate different metabolic pathways of (+) reticuline which would be converted into tembetarine in some species or into protoberberine and protopine alkaloids in others such as *F. coco*. The finding that (+) tembetarine chloride is an inefficient precursor of protopine in *Dicentra spectabilis*[9] would support the previous hypothesis.

EXPERIMENTAL

Melting points were determined with a Fisher–Johns block and are uncorrected. NMR spectra were recorded with a Varian A-60 spectrometer using TMS as the internal standard. UV spectra were recorded with a Beckman DK-2 spectrophotometer in EtOH 96%. Samples were counted in a Packard Tri-Carb model 93320 liquid scintillation spectrometer, in the usual scintillation solutions. Solvents were removed under diminished pressure below 50°. Microanalyses were performed by A. Bernhardt Laboratory, W. Germany.

Administration of the tracer and isolation of palmatine (I) and berberine (II). Sodium formate-^{14}C (1·3 mg, $9{\cdot}25 \times 10^{10}$ dis/min/mM) was administered to one intact medium size *F. coco* plant (about 2 m in height). The plant was growing out-of-doors in La Calera mountains in the province of Córdoba. The plant was harvested after 12 days, dried in a ventilated oven, and ground in a mill. The dried powder (700 g) was extracted first with ligroin, and then with MeOH until negative alkaloid reaction in the extract. The residue obtained for evaporation of the methanolic extract was dissolved in 0·1 N HCl and filtered. The acid extract was washed with Et_2O, made basic with NH_4OH to pH 10, and extracted with $CHCl_3$. The residue obtained for evaporation of the dried ($MgSO_4$) extract (1·9 g, $0{\cdot}27 \times 10^4$ dis/min/mg) was chromatographed on a column of acid alumina (Fluka, grade I);[10] elution with $CHCl_3$ and $CHCl_3$–MeOH (99:1) allowed the collection of the yellow fluorescent band. This fraction (18·5 mg, $0{\cdot}56 \times 10^4$ dis/min/mg) was a mixture of palmatine and berberine (TLC). The mixture was resolved into its components by preparative TLC on alumina (Merck, PF_{254}, type E). Palmatine was crystallized as the chloride (11·8 mg, m.p. 203–205°, $1{\cdot}14 \times 10^6$ dis/min/mM). The radioactive product (10 mg) was diluted with inactive material[11,12] (90 mg), and the mixture was recrystallized from EtOH–H_2O until constant activity yielding 83·5 mg of $1{\cdot}17 \times 10^4$ dis/min/mM.

8-Phenyldihydropalmatine (III). Radioactive palmatine chloride (80 mg) was dried *in vacuo* over P_2O_5 at 110° for 3 weeks. The anhydrous product was suspended in dry Et_2O (1·5 ml), 1 M phenylmagnesiumbromide (7 ml) was slowly added, and the mixture was stirred and heated under reflux in N_2 atmosphere for 7 hr, and then at room temperature for 24 hr. H_2O and 6 N HCl were added to the reaction mixture, and the solid product thus formed was extracted with 6 N HCl (50 ml). The acid solution was cooled on ice, made basic with NH_4OH, and the precipitate filtered off and dried. The yellow solid (50 mg, $1{\cdot}02 \times 10^4$ dis/min/mM) was recrystallized from EtOH to pure III, m.p. 158–160°, λ_{max}: 387 and 283 nm (log ϵ: 4·11 and 4·22); δ 2·80

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(broad signal, 2H, H-5), 3·40 (broad signal, 2H, H-6), 3·51 (s, 3H, O-Me), 3·75 (s, 6H, O-Me), 3·83 (s, 3H, O-Me), 5·73 (s, 1H, H-8), 6·03 (s, 1H, H-13), 6·65–7·45 (m, 9H, aromatic protons); specific activity: $1·03 \times 10^4$ dis/min/mM).

An inactive sample was analyzed. (Found: C, 75·67; H, 6·49; N, 3·43; $C_{27}H_{27}NO_4$ required: C, 75·50; H, 6·33; N, 3·26%.)

Oxidation of 8-phenyldihydropalmatine. Compound III (33 mg) was treated in the usual manner with CrO_3 (4 g) in 10% H_2SO_4 (12 ml). The benzoic acid thus produced was purified by repeated sublimation (60°, 10^{-3} torr) yielding 3 mg, m.p. 121°, spec. act. $0·22 \times 10^4$ dis/min/mM.

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