Biosynthesis of Tetrahydroisoquinoline Alkaloids in *Carnegiea gigantea* Br. & R.*

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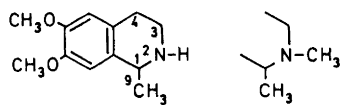
Recent reinvestigations [1,2] of the alkaloid content of the giant cactus or saguaro, *Carnegiea gigantea* Br. & R. (synonym: *Cereus giganteus* Engelm.), have shown the presence of, besides the previously [3] isolated carnegine (*2*), two other tetrahydroisoquinoline alkaloids, *viz.* the earlier from *Salsola* sp.[1] known salsolidine (*1*) and gigantine, the structure of which is being further studied.[4] 4-Hydroxy-3-methoxyphenethylamine (*3*: R_1=CH_3O: R_2=OH) and 3,4-dimethoxyphenethylamine (*3*: R_1=R_2=CH_3O) have been identified as trace constituents of the alkaloid fraction,[1] the major alkaloids being salsolidine and carnegine.



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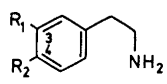

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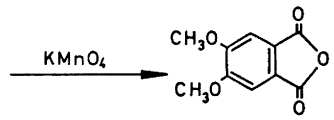
Simple tetrahydroisoquinolines in cacti, such as lophocerine,[5,6] pilocereine,[6] anhalamine,[7,8] and pellotine,[8,9] have been shown to originate from tyrosine. The only available detailed biosynthetic information concerns the peyote alkaloids pellotine and anhalamine.[7–9] These compounds are formed from tyrosine with 4-hydroxy-3-methoxyphenethylamine and 3-hydroxy-4,5-dimethoxyphenethylamine being two major intermediates.

We have now studied the biosynthesis of carnegine and salsolidine in young *C. gigantea* plants. The results obtained with labelled precursors are summarized in Table 1. The incorporation percentages should be considered as approximate, especially as regards experiments 2, 4, and 5, which were carried out with larger cacti than in the other experiments. The location of the label at the appropriate carbon atom in the derived alkaloid was, whenever feasible, determined by degradation procedures to prove a direct incorporation of a precursor. Alkaline permanganate oxidation [10] as well as Kuhn-Roth oxidation were used to degrade alkaloids derived from α,β-^{3}H or α-^{14}C-labelled phenethylamines and related amino acids, yielding in the first case inactive *m*-hemipinic anhydride (*4*) (Scheme 1), and by Kuhn-Roth oxidation likewise inactive acetic acid.



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Scheme 1.

The incorporation figures in Table 1 indicate that some features of the peyote alkaloid pathway may be valid also for *Carnegiea gigantea*. There are apparently, as in peyote, alternative routes operating from tyrosine *via* tyramine or *via* DOPA to dopamine. The reported presence [11] of large amounts of dopamine in the cactus suggests that this amine is a key compound in the biosynthesis of the *C. gigantea* alkaloids. A higher incorporation percentage might thus be expected, but there must also be a considerable dilution of the labelled precursor by endogenous dopamine. This probably accounts for the observed low values. Comparison of experiments 2, 4, and 5 suggests that at least one hydroxy group is needed for incorporation.

Zeisel demethylation of salsolidine from the feeding of methionine-methyl-^{14}C afforded radioactive tetramethylammonium iodide, carrying 99 % of the activity of the isolated alkaloid. Thus, the methyl group

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Table 1. Incorporation of radioactive compounds into carnegine and salsolidine.

Expt. No.	Precursor introduced	Amount fed		Incorporation rate ($\% \times 10^{-2}$)	
		mg	μCi	Carnegine [a]	Salsolidine [a]
13	DL-Tyrosine-α-^{14}C	0.6	50	0.18	0.85
11	Tyramine-α-^{14}C	2.1	50	0.08	0.19
16	DL-DOPA-α-^{14}C	2.5	50	0.09	0.06
14A	Dopamine-α,β-^{3}H	0.0003	50	0.30	0.42
5	3-Hydroxy-4-methoxy-phenethylamine-α,β-^{3}H	2.3	20	—[b]	0.29[c]
4	4-Hydroxy-3-methoxy-phenethylamine-α,β-^{3}H	5.0	56	—[b]	1.12[c]
15	3,4-Dimethoxy-phenethylamine-α-^{14}C	0.58	25	0.22	0.41
2	3,4-Dimethoxy-phenethylamine-α-^{14}C	0.7	30	—[b]	<0.06[c]
12A	L-Methionine-methyl-^{14}C	0.31	25	0.17	0.87
12B	L-Methionine-methyl-^{14}C	0.31	25	0.84	4.86

[a] After addition of 100 mg inactive carrier material.
[b] Carnegine not isolated.
[c] Experiments 2, 4 & 5 were performed with considerably larger cacti than in the other cases. No carrier was added in these experiments.

of methionine serves as a precursor of the *O*-methyl groups, but not of the C-1 and C-9 positions (*cf. 1*). This is in agreement with the biosynthesis of pellotine,[9] which also has a methyl group attached to C-1. Further work will be needed to clarify the ring-closing mechanism and the interconversions of the alkaloids. Such a study is now in progress.

Experimental. Feeding of precursors and isolation of alkaloids. The labelled precursors were injected into the cacti, which in experiments 2, 4, and 5 were grown outdoors in their native habitat in Arizona. In other experiments cacti were cultivated under controlled greenhouse conditions. Three to four weeks later the alkaloid fractions were isolated and carnegine and salsolidine were isolated by thin layer or column chromatography. The alkaloids were recrystallized as the hydrochlorides to constant specific activity from ethanol ether, usually after the addition of 100 mg inactive carrier material. The alkaloids isolated in experiment 15 were found to be highly radioactive, contaminated with fed labelled 3,4-dimethoxyphenethylamine. This could, however, be removed by preparative TLC.

Oxidation of salsolidine (Scheme 1). Salsolidine HCl (47 mg) was dissolved in water (2 ml) and to the solution was added 2 M KOH (0.2 ml) and potassium permanganate (100 mg). The mixture was heated under reflux for 4 h. After cooling, the mixture was acidified and the manganese dioxide dissolved by dropwise adding conc. sulphuric acid and a saturated solution of sodium sulphite.[10] The resulting mixture was extracted with ether (3 × 25 ml). The ether extract was evaporated to dryness and the residue purified by sublimation *in vacuo* (0.1 mmHg). The yield of *m*-hemipinic anhydride (*4*) after recrystallization in benzene-light petroleum was 9 mg (22 %), m.p. 174–7°. Reported[12] m.p. 175°.

Zeisel demethylation of salsolidine. Salsolidine HCl (10 mg) was refluxed in a mixture of hydriodic acid (2.0 ml; 57 %) and acetic anhydride (1.3 ml).[6] A stream of nitrogen was passed through the solution. The distilling methyl iodide was collected in a solution of 10 % trimethylamine in absolute ethanol (10 ml). After 20 min the crystalline precipitate of tetramethylammonium iodide (4 mg; 25 %) was filtered off and measured for radioactivity.

Kuhn-Roth oxidation of salsolidine. Salsolidine. Salsolidine HCl (45 mg) was heated under reflux for 90 min in a mixture of chromium trioxide (3.3 g), conc. sulphuric acid (5 ml), silver sulphate (100 mg) and water (20 ml). The acetic acid formed was recovered by steam distillation and potentiometrically titrated to pH 8.5 with 0.1 N sodium hydrox-

ide. The titrated solution was evaporated to dryness and sodium acetate (8.5 mg; 56.5 %) recrystallized from ethanol-ether.

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On the Existence of the 1,3,5,7-Tetramethyl-2,4,6,8-tetrathiaadamantane Dianion

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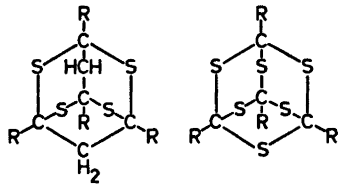
A solution of 1,3,5,7-tetramethyl-2,4,6,8-tetrathiaadamantane (I)* in tetrahydrofuran (THF) was recently reported as developing a yellow colour, when stirred with sodium-potassium alloy (Na-K).[1,2] The colour was ascribed to a dianion, formed by addition of two electrons to the otherwise unchanged I. This conclusion was based mainly on the following observations. The yellow solution gave no electron spin resonance signal, reduced quinones, and could be titrated with iodine, indicating a 4 % conversion of I into dianion after 30 min at 10°. Unlike alcohols, stronger acids caused immediate discoloration. After quenching, no organosulphur compound other than I could be detected. A multi-cycle voltammogram of I at a mercury electrode was interpreted as two reversible reduction waves.

When exposed to Na-K, tetramethylhexathiaadamantane (II) was changed to a stinking mixture of organosulphur compounds, believed to indicate extensive reductive cleavage of C—S bonds.[2] In the present note, we wish to present evidence suggesting similar cleavage in the formation of the yellow species from I. On the other hand, we were unable to reduce I electrochemically.



I II

* Throughout the present paper, R = CH_3.