**Tetrahedron Letters No. 38, pp 3401 - 3404, 1976. Pergamon Press. Printed in Great Britain.**

# LOSS OF TRITIUM DURING THE BIOSYNTHESIS OF METELOIDINE AND SCOPOLAMINE FROM [N-METHYL-$^{14}C$, 6β,7β-$^{3}H_2$]TROPINE

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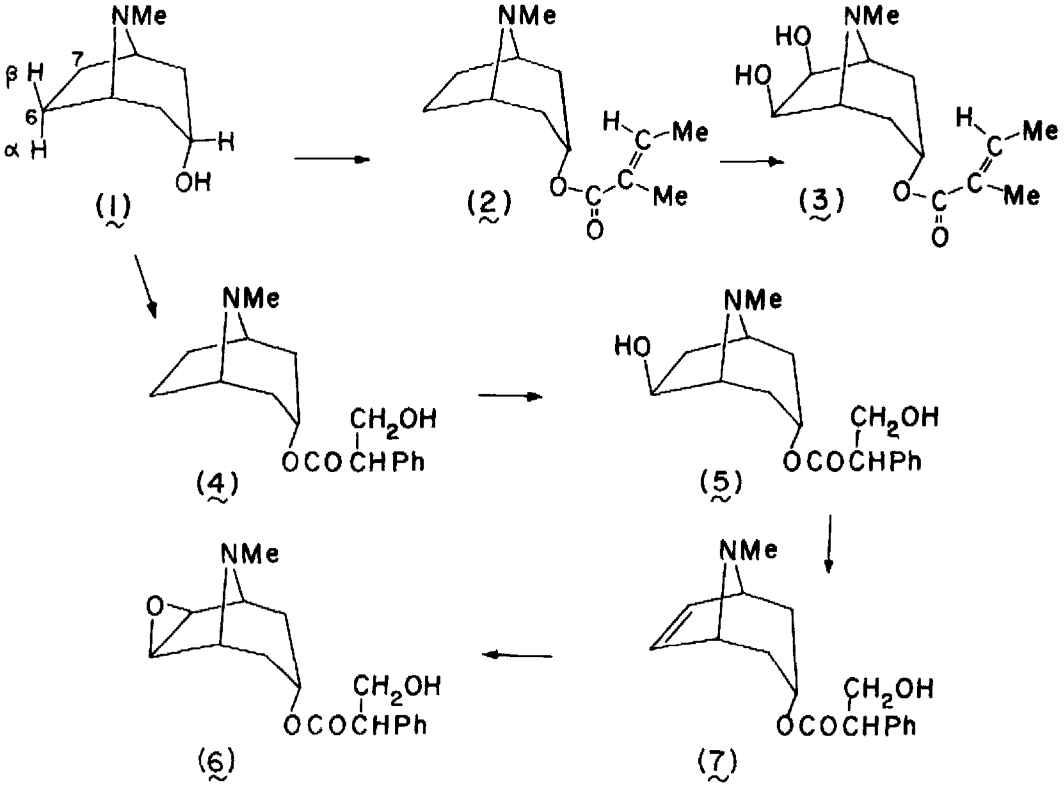
**(Received in USA 14 July 1976; received in UK for publication 9 August 1976)**

We have previously established[2] that tropine (1) is a precursor of meteloidine (3), hydroxylation of the tropine moiety apparently occurring after the formation of its tigloyl ester (2)[3,4]. It is also known that hyoscyamine (4), the tropic acid ester of tropine, is a precursor of scopolamine (6), and early



work with non-radioactive substrates favored the formation of the epoxide via 6β-hydroxyhyoscyamine (5) and 6,7-dehydrohyoscyamine (7)[5]. We have now examined the stereochemical course of these oxidations of tropine by feeding to *Datura* species [6β,7β-$^3H_2$]tropine, which was prepared by the following method. Scopolamine was converted to 6,7-dehydrohyoscyamine by Sharpless's method[6]: n-Butyl lithium (35.7 mmol, 2.1M in hexane) was added with rapid stirring to a suspension of $WCl_6$ (5.48 g, 13.8 mmol) in THF (40 ml) at -78° under $N_2$ . After 5 minutes the reaction mixture was allowed to warm to 20°, and a solution of scopolamine (3.9 mmol) in THF (10 ml) added during 5 minutes. After stirring for 30 minutes the reaction mixture was added to $H_2O$ (150 ml) containing NaOH (5M) and sodium potassium tartrate (1M). The solution was extracted with $CHCl_3$, dried ($Na_2SO_4$), and evaporated to yield (7) as a pale yellow oil (870 mg, 3.03 mmol), having a mass spectrum, m/e 287.1539, calc. for $C_{17}H_{21}NO_3$: 287.1521; 138.0927 (M-tropic acid moiety), calc. for $C_8H_{12}NO$: 138.0918. Hydrogenation in ethyl acetate with $D_2$ in the presence of 10% Pd/C afforded hyoscyamine. Hydrolysis of this deuterated material yielded tropine which was oxidized to tropinone. The $^1H$-NMR spectrum of this compound indicated that the deuterium was located in the 6β and 7β (*exo*) positions[7]. Hydrogenation of (7) with tritium under the same conditions thus afforded [6β,7β-$^3H_2$]hyoscyamine which on hydrolysis yielded [6β,7β-$^3H_2$]tropine, having the same specific activity, indicating that all the tritium was in the tropine moiety[8]. This [$^3H$]tropine was mixed with [N-*methyl*-$^{14}C$]tropine[2] yielding material having a $^3H/^{14}C$ ratio of 7.3. Further confirmation of the location of the tritium was obtained by oxidizing this doubly labeled material with $CrO_3$ in sulfuric acid[9], when N-methylsuccinimide ($^3H/^{14}C = 7.2$) was obtained.

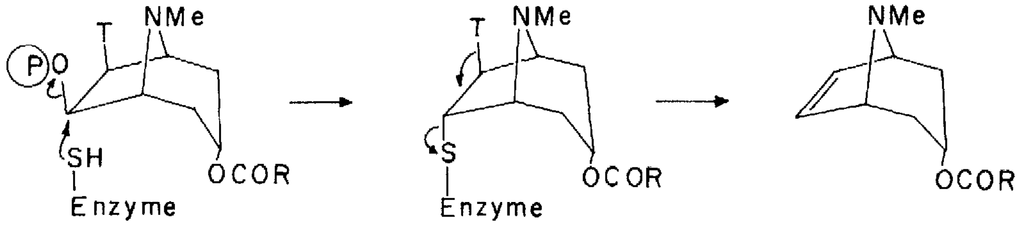
The [N-*methyl*-$^{14}C$, 6β,7β-$^3H_2$]tropine was fed to *Datura innoxia* and *D. meteloides* by the wick method for 7 days, and the alkaloids isolated as previously described[2]. Their activities are recorded in the Table. As expected, the hyoscyamine and re-isolated tropine had $^3H/^{14}C$ ratios essentially the same as the administered tropine. However the meteloidine and scopolamine retained only small amounts of tritium. Thus the di-hydroxylation of the tropine moiety of (2) proceeds with retention of configuration. This result is typical of direct hydroxylations at saturated

Table

| | *Datura innoxia* | *Datura meteloides* |
|---|---|---|
| [N-methyl-$^{14}$C, 6β,7β-$^{3}H_2$]Tropine | | |
| Amount fed | 0.5 mmol | 0.5 mmol |
| Specific activity* | $5.63 \times 10^7$ | $5.63 \times 10^7$ |
| $^3H/^{14}C$ | 7.3 | 7.3 |
| Fresh weight of plants (g) | 587 | 1200 |
| Hyoscyamine | | |
| Weight (mg) | 123 | 43 |
| Specific activity* | $3.22 \times 10^5$ | $1.74 \times 10^4$ |
| Specific incorporation (%) | 0.57 | 0.031 |
| $^3H/^{14}C$ | 6.3 | 7.7 |
| Scopolamine | | |
| Weight (mg) | 66 | 133 |
| Specific activity* | $1.04 \times 10^5$ | $1.72 \times 10^4$ |
| Specific incorporation (%) | 0.18 | 0.030 |
| $^3H/^{14}C$ | 0.3 | 0.5 |
| Meteloidine | | |
| Weight (mg) | 53 | 64 |
| Specific activity* | $1.09 \times 10^5$ | $3.4 \times 10^5$ |
| Specific incorporation (%) | 0.19 | 0.60 |
| $^3H/^{14}C$ | 0.4 | $<0.1$ |
| Tropine | | |
| Weight (mg) | not isolated | 3.5 |
| Specific activity* | | $1.47 \times 10^7$ |
| Specific incorporation | | 26.1 |
| $^3H/^{14}C$ | | 7.5 |

* $^{14}C$ activity, dpm/mmol.

carbon atoms[10] . If the previous work[5] on the biosynthesis of scopolamine is accepted, the present results indicate that the formation of 6,7-dehydrohyoscyamine from 6β-hydroxyhyoscyamine involves a cis-dehydration. Olefin formation catalyzed by dehydratases usually involve the trans removal of the elements of water[11], however some cis-dehydrations have been observed in Nature, for example the formation of 5-dehydroshikimic acid from 5-dehydroquinic acid[12]. The dehydration could of course proceed by a two step mechanism as illustrated below:



This investigation was supported by a research grant GM-13246 from the National Institutes of Health, U. S. Public Health Service.

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