PREPARATION OF TRITIUM LABELLED SYNTHANECINE A AND ITS BIS-N-ETHYLCARBAMATE

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SUMMARY

A procedure is described for incorporating tritium into the 3-CH_2 side chain of synthanecine A, and preparing the carbamate, 2,3-bis-N-ethylcarbamoyloxymethyl-1-methyl-3-pyrroline, a hepatotoxic pyrrolizidine alkaloid analogue. The pyrrolizidine amino alcohol, retronecine, can be tritium labelled in a similar way.

Keys words: Tritium, synthanecine A, pyrrolizidine, hepatotoxic, retronecine, N-ethylcarbamate.

Synthanecine A (2,3-bishydroxymethyl-1-methyl-3-pyrroline) (1)[1] is a synthetic analogue of the amino alcohol moieties (necines) of many toxic pyrrolizidine ester alkaloids. Thus, some of its esters, especially its bis-N-ethycarbamate (2,3-bis-N-ethylcarbamoyloxymethyl-1-methyl-3-pyrroline) (4) have toxicity in animals qualitatively similar to that of hepatotoxic pyrrolizidine alkaloids such as monocrotaline[2]. The distribution of tritium labelled synthanecine A 2,3-bis-N-ethyl carbamate has been studied in rats[3], and compared with that of the similar, semisynthetic alkaloid, ^{3}H-retronecine bis-N-ethyl-carbamate[4].

For tritium labelling, pure synthanecine A[1] was oxidized to the unstable 3-aldehyde (2), which was reduced back to the alcohol (3) using ^{3}H-sodium borohydride[3]. The key step is the oxidation, which requires a specially prepared manganese

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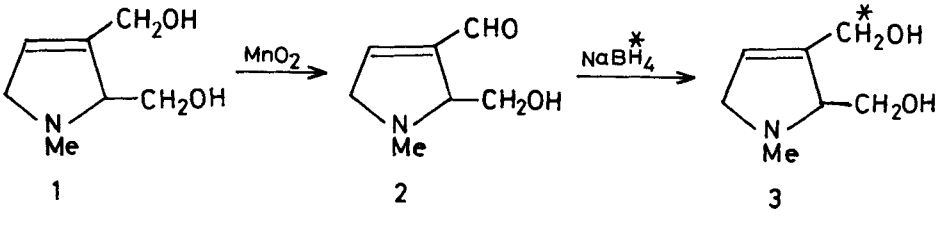
dioxide[5]. The procedure, of which a brief account was given previously[3], is here described in detail. The manganese dioxide has been improved by admixture with an inert support (Hyflo supacel).

Synthanecine A reacts rapidly with ethyl isocyanate to give the bis-N-ethylcarbamate (4)[1] when the basic catalyst 1,4-diazabicyclo-[2,2,2]octane (trimethylenediamine) is present. Lengthy reaction times lead to troublesome impurities, and should be avoided; this is in contrast with the preparation of retronecine bis-N-ethylcarbamate, which requires up to 6 hours for completion[4]. After the reaction, the catalyst is easily removed by sublimation under reduced pressure. The product purity is good, being similar to that of the starting material; however if necessary both synthanecine A and its bis-N-ethylcarbamate can be purified via their crystalline picrolonates[1].

In the procedure described below, the radioactive synthanecine was diluted with unlabelled material at two stages since a product with low specific activity was required. If higher activity is required, the second dilution can be omitted



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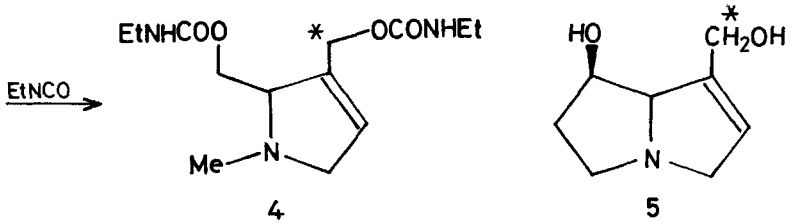

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and the first reduced; however if the amino alcohol is not first diluted before extraction from the aqueous reaction mixture, the yield of tritiated product may be lower.

The procedure for preparing 9-^{3}H-retronecine (5) from retronecine, previously described briefly[4], is essentially the same as that for ^{3}H-synthanecine A. As well as the N-ethylcarbamates, other potentially toxic esters of the tritiated amino alcohols can be prepared by standard methods[1,6,7]. The availability of a route to macrocyclic retronecine diesters[8] now enables specifically labelled compounds closely similar to natural pyrrolizidine alkaloids to be made.

EXPERIMENTAL

Chemicals, of analytical grade where available, were from BDH Chemicals Ltd or Aldrich Chemical Co Ltd. Tlc was as previously described[1,3]; radioactivity was detected using a Packard 7200 radio-chromatogram scanner.

Manganese dioxide[5]. To a solution of manganese (II) sulphate (8.5 g) in water (100 ml), stirred at 0° (ice-water bath) was added Hyflo supacel (5 g) (Johns Manville Co Ltd), then, during 2 min, a solution of potassium permanganate (5 g) in water (100 ml) at 15-20°. After being stirred at 0-5° for 1 h, the solids were filtered off (pump) and surplus liquid pressed out. The residue was resuspended in water (100 ml), filtered and pressed as before, then dried overnight at approx. 120°, to give dark brown lumps (12 g) which had a moderately acid reaction when moistened. The product retained its oxidative activity for at least a year when stored in a closed bottle at room temperature.

^{3}H-Synthanecine A (3). The above mixture of manganese dioxide and Hyflo supacel (2.2 g) was finely powdered, then stirred at room temperature with a solution of pure synthanecine A[1,7] (205

mg) in chloroform (27 ml). The solution was filtered (pump) and shaken with 2 lots (each 6 ml) of ice cold citrate-phosphate buffer (0.1 M), pH 3.5. The combined aqueous extract was washed twice with its own volume of butan-1-ol, then 3 times with ether, almost saturated with K_2CO_3 (ice cooling), and extracted with chloroform (3 x 10 ml). The combined chloroform extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give the 3-aldehyde (2) as a colourless gum (27 mg, 13%); IR and ^{1}H-NMR as previously reported[5]. This unstable product was dissolved in water (1 ml) and ^{3}H-sodium borohydride (approx. 3 mg, 21 mCi; Amersham International Ltd) was added at room temperature, mainly as solid, the remainder being washed in with a few drops of water. After 30 min at room temperature, unlabelled sodium borohydride (7 mg in 0.5 mg H_2O) was added. After a further 30 min, unlabelled synthanecine A (100 mg) was dissolved in the mixture, which was then saturated with potassium carbonate (cooling) and extracted with 4 lots of chloroform (4 ml each). (Note that the $CHCl_3$ is the upper layer). The combined extracts were dried (Na_2SO_4) and concentrated, first in a stream of dry N_2 and then under reduced pressure to give an almost colourless gum (114 mg, 90% chemical recovery; 13.06 mCi, approx. 62% radiochemical yield), identical (IR, NMR, tlc) with synthanecine A[1].

^{3}H-Synthanecine A bis-N-ethylcarbamate (4). The above ^{3}H-synthanecine A (31.0 mg, 3.55 mCi), unlabelled synthanecine A (71.5 mg) and 1,4-diazabicyclo[2,2,2]octane (5 mg) were dissolved in freshly redistilled ethyl isocyanate (2.5 ml), and the solution was heated under reflux for 5 min. Excess reagent was removed in a stream of N_2 and the residue dissolved in water (3 ml). The solution was acidified (HCl), washed with ethyl acetate (4 x 4 ml), basified with ammonia solution and extracted

with ethyl acetate (4 x 5 ml). The combined basic extracts were dried (Na_2SO_4) and evaporated, first in a stream of N_2, then at 100° under reduced pressure, leaving the product as a gum (180 mg; 88%; 3.0 mCi, 85% radiochemical yield), specific activity 4.745 mCi/mmol. The ^1H-NMR and IR spectra were identical with those described for unlabelled synthanecine A bis-N-ethyl-carbamate[1], and tlc gave a single spot, coinciding with a single peak of radioactivity.

REFERENCES

1. A. R. Mattocks, Nature, 232, 476 (1971).
2. A. R. Mattocks, J. Chem. Soc., Perkin I, 707 (1974).
3. A. R. Mattocks and I. N. H. White, Chem.-Biol. Interact., 15, 173 (1976).
4. A. R. Mattocks, Xenobiotica, 7, 665 (1977).
5. A. R. Mattocks, J. Chem. Res. (S), 40 (1977).
6. A. R. Mattocks, J. Chem. Soc. (C), 2698 (1969).
7. A. R. Mattocks, J. Chem. Soc., Perkin I, 896 (1978).
8. D. J. Robins and S. Sakdarat, J. Chem. Soc. Chem. Commun., 282 (1980).