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Synthesis of Tritium Labelled Metaraminol, α-Methylnoradrenaline and their corresponding β-Desoxyderivatives

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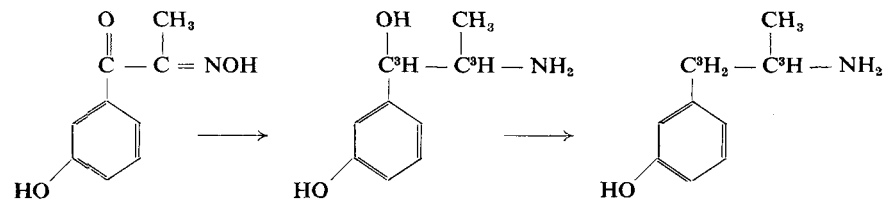
SUMMARY

m-hydroxy-α-oximinopropiophenone was hydrogenated by tritium gas in the presence of Pd on carbon catalyst. The crude product was chromatographed on a cation-exchange column. Two major peaks were obtained. Subsequent radio-paper chromatography revealed that these peaks were identical with metaraminol and α-methyl-m-tyramine respectively. When 3,4-dibenzyloxy-α-oximinopropiophenone was used as starting material both erythro and threo α-methylnoradrenaline as well as α-methyldopamine were obtained.

INTRODUCTION.

Analogues of the neurohumoral transmitter substance, noradrenaline have been shown to be valuable tools in the investigation of adrenergic mechanisms. Among these analogues the α-methylated compounds are of particular interest. Although there are methods for the fluorimetric determination of these amines, the use of labelled compound is more convenient and permits work at lower concentrations [1]. Further, a radioisotope label facilitates the search for metabolites.

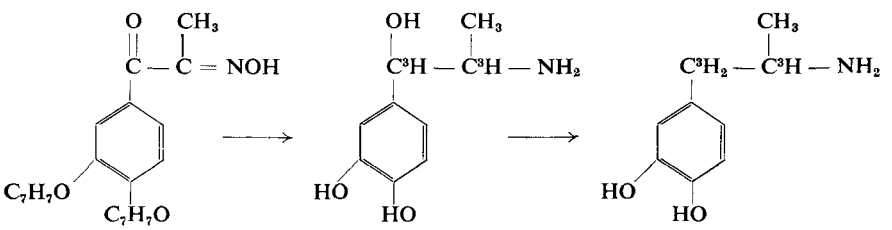
In the present work we have tritiated *m*-hydroxy-α-oximinopropiophenone and 3,4-benzyloxy-α-oximinopropiophenone by the method of Birkhofer and Hempel [2] in order to get ^{3}H-metaraminol and ^{3}H-α-methylnoradrenaline respectively. By the same method it should also be possible to get the respective β-desoxy derivatives α-methyl-*m*-tyramine and α-methyldopamine. The following reaction schemes are proposed :



m-hydroxy-
-α-oximino-
propiophenone

metaraminol

α-methyl-
-*m*-tyramine



3,4-dibenzyloxy-
-α-oximino-
propiophenone

α-methyl-
noradrenaline

α-methyl-
dopamine

Any tritium bound to oxygen or nitrogen will be washed away in a subsequent separation step on an ion-exchange column.

Experimental and results.

Synthesis of m-hydroxy-α-oximinopropiophenone.

m-hydroxypropiophenone was dissolved in HCl-saturated, ice-chilled dry ether and *n*-butylnitrite was added in portions with stirring during a period of 60-90 min. The solution was left over night at room temperature and was then extracted several times with diluted sodium hydroxide. After acidifying the extract the oily crude product precipitated and was extracted with ether. The ether was evaporated and the residue washed several times with benzene and recrystallized from water (Mp 138° C). Lit. value 138° C [3].

Synthesis of 3,4 *dibenzyloxy-α-oximinopropiophenone.*

3,4-dihydroxypropiophenone was benzylated with benzylchloride in absolute ethanol in the presence of potassium carbonate. The benzylated compound was recrystallized from ethanol and petrol ether (Mp 64° C). It was then converted to the corresponding oximino-derivative as described above for the *m*-hydroxy compound [3]. Recrystallized from benzene and petrol ether it melted at 111° C.

Catalytic tritiation.

0.1 mM of either of the two intermediates described above was dissolved in ethanol containing 3 per cent (w/w) HCl gas and about 100 mg of Pd on carbon (10 %) catalyst was added. The reaction mixture was placed in the tritiation apparatus according to Birkhofer and Hempel [2] and was exposed to 2-5 C tritium gas for 3-4 hrs. After separation from excess tritium the catalyst was removed from the reaction mixture by filtering through silica gel. The solvent was then evaporated under reduced pressure at about 25° C.

Ion-exchange chromatography.

The crude product was dissolved in 2 ml of water. After addition of 2 mg ascorbic acid and 20 mg EDTA, adjusted to pH 6.5 by means of potassium carbonate, the mixture was passed through a cation-exchange column (Dowex 50W X4, diameter, 4 mm, height, 400 mm and buffered at pH 6.5). The column was thoroughly rinsed with water and then eluted with N HCl. The acid was put through the column with the aid of a micro-flow pump at a constant rate of 12 ml/hr. The effluent was passed through a flow-cell scintillation detector containing antracene crystals. The relative radioactivity was measured by a rate-meter and recorded against time by a potentiometer recorder. A time-operated fraction collector picked up all 3-ml fractions so that each 1/2 inch on the recorder paper represented 1 fraction. After 70-80 fractions the elution was discontinued.

When *m*-hydroxy-α-oximinopropiophenone was used as starting material the ion-exchange chromatogram showed two distinct peaks, A_1 and B_1 (Fig. 1). When, on the other hand, the starting material was 3,4-dibenzyloxy-

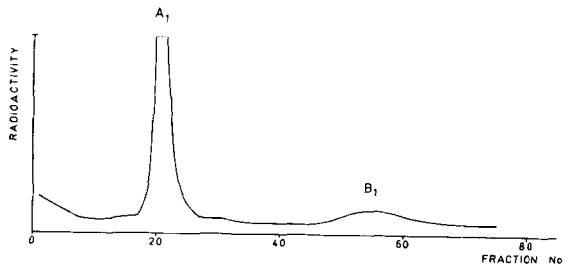


FIG. 1. Ion-exchange chromatography of the crude product obtained after catalytic tritiation of *m*-hydroxy-α-oximinopropiophenone. After application of the material to the column (Dowex 50 W X4, diameter, 4 mm, height, 400 mm and buffered at pH 6.5) and subsequent washing with water elution was performed with N HCl. The radioactivity of the eluate was recorded continuously with the aid of a flow-cell scintillation detector and a ratemeter. All 3 ml fractions were collected by a fraction-collector.

α-oximinopropiophenone four distinct peaks, A_2, B_2, C_2 and D_2, could be observed (Fig. 2). In addition there seemed to be two minor peaks in front of the two peaks A_2 and B_2 respectively.

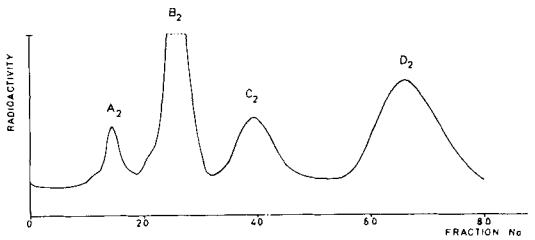


FIG. 2. Ion-exchange chromatography of the crude product obtained after catalytic tritiation of 3,4-benzyloxy α-oximinopropiophenone. For further explanation see Fig. 1.

Identification of the radioactive compounds.

Ten microlitres of each of the fractions containing radioactivity, as indicated by the ion-exchange radio-chromatograms, were chromatographed on Munktell 302 S paper together with reference substances. The following solvent systems were used : (*a*) Isopropanol : 2 N HCl, 65 : 35, v/v, foi 18 hrs. (*b*) Pyridine : acetic acid : methylethylketone : water, 1 : 2 : 10 : 3, v/v for 3 hrs. After development of the spots with diazotized *p*-nitroaniline the chromatograms were scanned for radioactivity in a gas-flow radio-paper chromatogram scanner.

The compounds under the peaks A_1 and B_1 had Rf values identical with metaraminol and α-methyl-*m*-tyramine respectively (Fig. 3). The peak D_2 appeared to consist of α-methyl-dopamine (Fig. 4). The compounds under B_2 and C_2 did not separate from α-methyl-noradrenaline in any of the solvent systems used (data not shown). For this reason the following experiment was designed. A sample from peak B_2 was mixed with authentic erythro α-methyl-noradrenaline and a sample from C_2 with authentic threo α-methyl-noradrenaline and then chromatographed on a DOWEX 50W X4 column (for details see Carlsson *et al.* [4]). Fluorescence and radioactivity of each fraction were measured (Fig. 5). The fluorescence of the radioactive compounds was negligible since the reference substances were in much larger concentrations. The radioactive elution pattern coincided with the fluorescence elution pattern of the respective authentic substances in both samples, Note that the position of the peak is different for the two diastereoisomers.

When tested according to the THI-method as described by Bertler, Carlsson and Rosengren [5], threo α-methylnoradrenaline yields a fluorescence of the same intensity as NA and 30-40 times stronger than erythro

α-methylnoradrenaline (Muscholl, personal communication). Aliquots of B_2 and C_2 (estimates of the concentrations were made by measuring the native fluorescence) and authentic erythro and threo α-methylnoradrenaline were

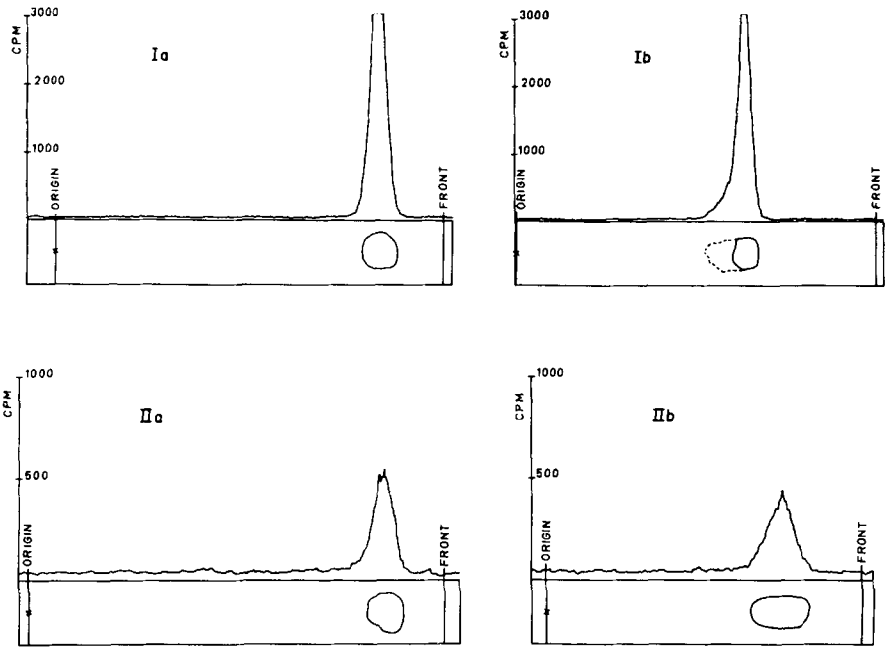


FIG. 3. Radio-paper chromatography of the compounds under A_1 and B_1. I. 10 μl of the eluate under A_1 were mixed with 5 μg of authentic metaraminol and chromatographed in solvent system (*a*) and (*b*) respectively (see text).

II. 10 μl of the eluate under B_1 were mixed with 5 μg of authentic α-methyl-*m*-tyramine and chromatographed in solvent system (*a*) and (*b*) respectively (see text).

After development of the spots with diazotized *p*-nitroaniline the radioactivity was scanned by a radio-paper chromatogram scanner.

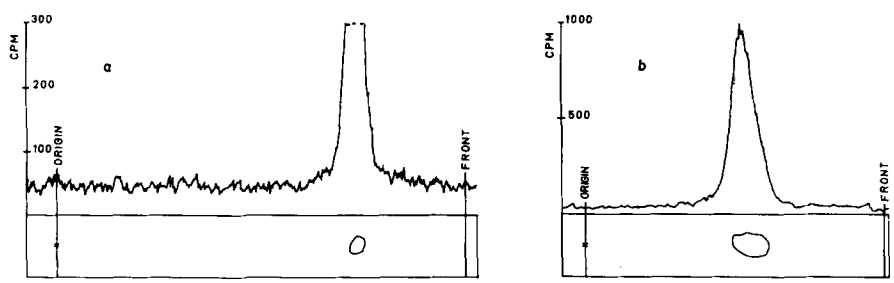


FIG. 4. Radio-paper chromatography of the compound under D_2. 10 μl of the eluate under D_2 were mixed with 5 μg of authentic α-methyldopamine and chromatographed in solvent system (*a*) and (*b*) respectively. After development of the spots with diazotized *p*-nitroaniline the radioactivity was scanned by a radio-paper chromatogram scanner.

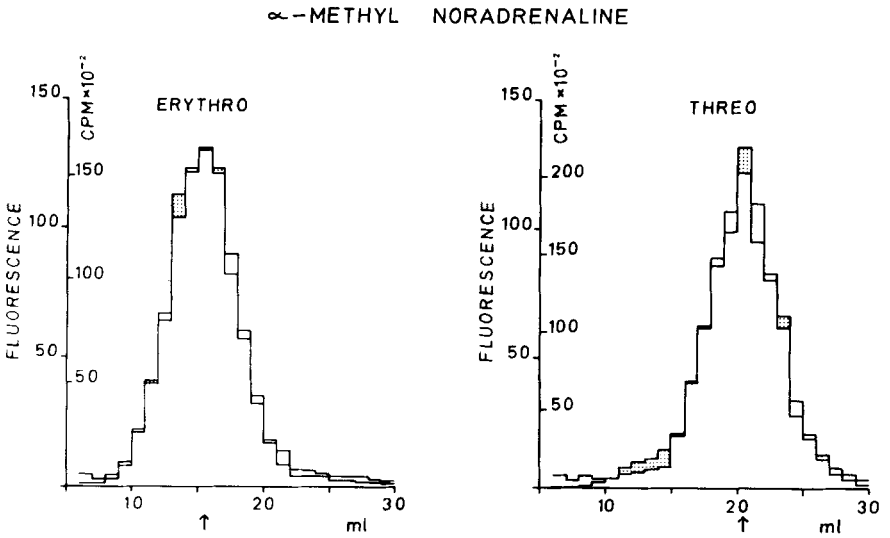


FIG. 5. Ion-exchange chromatography of the compounds under B_2 and C_2. An aliquot of B_2 was mixed with 25 μg authentic erythro α-methylnoradrenaline and an aliquot of C_2 was mixed with 25 μg authentic threo α-methylnoradrenaline. The samples were put on Dowex 50W X4 columns. After washing with water the columns were eluted with N HCl. From 7 ml all 1 ml fractions were collected and their fluorescence and radioactivity measured. Shaded area : The radioactive elution pattern exceeds the fluorescence elution pattern.

TABLE 1. Fluorescence characteristics of the compounds under B_2 and C_2. 0.2 μg of each of the compounds listed were treated according to the THI-method. The fluorescence was measured at 400 and 500 nm activating and fluorescence wavelength respectively.

Compound	Fluorescence arbitrary units
Noradrenaline	50
Erythro α-methylnoradrenaline	3
Threo α-methylnoradrenaline	43
Compound B_2	4
Compound C_2	36
Reagent blank	2

treated accordingly. For comparative purposes also NA was included in the test (Table 1). Only the sample from C_2 and threo α-methylnoradrenaline gave a fluorescence intensity of the same order of magnitude as that of NA. Thus the following criteria argue for B_2 and C_2 being identical with erythro and threo α-methylnoradrenaline respectively :

(1) Identical Rf values in two solvent systems.
(2) The same elution pattern on a Dowex 50 ion-exchange column.
(3) Identical fluorescence characteristics after treatment according to the THI-method.

The peak A_2 has so far not been identified.

Yield and specific activity.

The molar yield of identified labelled amines was in general about 40 per cent of the charge, ^{3}H-metaraminol and ^{3}H-α-methylnoradrenaline being the main products.

The approximate specific activities obtained were for metaraminol 120 and for α-methylnoradrenaline 60 mC/mM. The specific activities of the desoxyderivatives were in general 3-5 times higher than that of their respective parent compounds *i.e.* 500 mC/mM for α-methyl-*m*-tyramine and 200 mC/mM for α-methyldopamine.

Discussion.

When *m*-hydroxy α-oximinopropiophenone was hydrogenated catalytically with tritium as described above, tritium labelled metaraminol and α-methyl-*m*-tyramine could be isolated. Treatment of 3,4-dibenzyloxy α-oximinopropiophenone in the same way yielded, besides α-methyldopamine, both the erythro and threo forms of α-methylnoradrenaline. Metaraminol, like α-methylnoradrenaline, has two asymmetric carbon atoms and therefore two diastereoisomers would be expected also of this compound. As only erythro metaraminol was available as reference material it was not possible to predict the exact position for the peak of the threo compound. The two diastereoisomers of α-methylnoradrenaline were easily separated on the ion-exchange column and therefore it does not seem very likely that two isomers of metaraminol should have the same migration rate. It is more probable that the peak of threo metaraminol will appear between those of erythro metaraminol and its desoxy derivative, in analogy with threo α-methylnoradrenaline. In fact a very low peak in this position may be distinguished from the chromatogram in Figure 1.

In order to concentrate the preparation of erythro α-methylnoradrenaline we first tried to evaporate the hydrochloric acid eluate to dryness under

reduced pressure at +30° C. We then found that the configuration was changed to the threo form. No such phenomenon could be observed for erythro metaraminol. It may be suggested therefore that during the catalytic tritiation mainly the erythro forms of metaraminol and α-methylnoradrenaline are formed. When dried in an acid medium (as for the treatment of the crude product) erythro α-methylnoradrenaline will be converted to its diastereoisomer. Such a change in the configuration would be less pronounced for metaraminol.

In biochemical work it is of interest to know the position of the label since metabolic conversion may alter the specific activity. α-methyl-*m*-tyramine is metabolically converted to metaraminol resulting in loss of one hydrogen atom in the 2′ position. If this hydrogen atom is replaced by tritium the conversion will result in a change in the specific activity. By the method used the tritium label should be in the positions 1′ and 2′ (see the reaction scheme above). It cannot be excluded, however, that tritium will be introduced into other sites in the molecule by exchange. This may explain why the specific activity of the α-methyl-*m*-tyramine is about four times higher than that of the metaraminol obtained from one and the same operation. In order to determine the relative labelling in 2′ position it is necessary to estimate the change in specific activity after β-hydroxylation biochemically, What has been said about α-methyl-*m*-tyramine may also be true of α-methyldopamine

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