

revealed precipitation of some radioactive compounds apart from the thyroglobulin. These compounds were, however, absent after purification of the thyroglobulin (Fig. 2). The purified hog thyroglobulin produced, however, no precipitation band with this anti-thyroglobulin containing human serum.

This method which combines fractionation on Sephadex G-200 column and the previously used purification on DEAE-cellulose offers some advantages as compared to other methods. Our own studies indicate a much more satisfactory preliminary purification with Sephadex than with ammonium sulphate hitherto used.

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1. Ui, N. and Tarutani, O. *J. Biochem. (Tokyo)* **50** (1961) 508.
2. Edelhoch, H. *J. Biol. Chem.* **235** (1960) 1326.

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Synthesis of 2-Phenylisopropylhydrazine-1-¹⁴C Hydrochloride

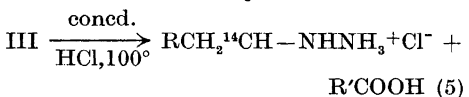
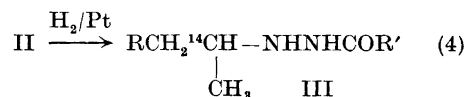
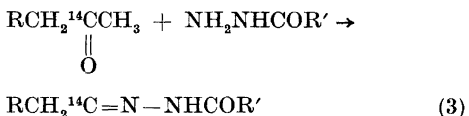
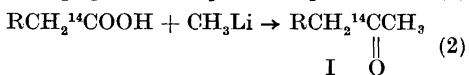
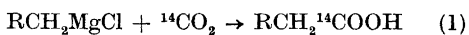
LENNART EBERSON

Department of Organic Chemistry, University of Lund, Sweden

Interest in organic hydrazine compounds is largely a result of their more or less pronounced inhibiting action upon the enzyme monoamine oxidase.^{1,2} Recently, it has also been observed that certain hydrazine derivatives possess cytostatic properties,³⁻⁶ possibly due to the low release of hydrogen peroxide known to occur when hydrazines undergo autoxidation.^{6,7}

2-Phenylisopropylhydrazine is one of the most potent monoamine oxidase inhibitors and has been used widely as an antidepressant drug. In order to make studies on the metabolism and distribution of this compound in biological systems possible a method for the synthesis of ¹⁴C labelled 2-phenylisopropylhydrazine has been developed. The method should be generally applicable to the synthesis of ¹⁴C labelled hydrazine derivatives of this type.

The synthesis includes reaction steps (1)–(5) (R = phenyl, R' = CH₂CH₂–CH(NH₂)COOH):



Labelled phenylacetone (I, R = phenyl) has been synthesized previously by the reaction between phenylacetyl chloride and ethyl ethoxymagnesiummalonate and subsequent hydrolysis and decarboxylation of the intermediate malonic ester,⁸ but this method was found to give consistently low yields of a rather impure product. Instead, phenylacetone was prepared by reaction of free phenylacetic acid with an excess of methyl lithium,⁹ which gives about 90% yield of a pure product. This method avoids the troublesome step of preparing the acid chloride.

Step (3) involves the preparation of the solid γ -glutamylhydrazone of phenylacetone, (II), which can then be catalytically hydrogenated using Adam's catalyst without any side reactions resulting from cleavage of the nitrogen to nitrogen bond. Under these reaction conditions it is not possible to avoid this side reaction if the unsubstituted phenylacetone hydrazone is

used.¹⁰ The last step (5) is the hydrolysis of the hydrazide (III), which can be effected by treatment with concentrated hydrochloric acid at 100° for 20 min. Kinetic studies on the hydrolysis of acet-hydrazide in acid solution¹¹ indicate that this treatment should give a practically complete conversion.

The over-all yield of recrystallized 2-phenylisopropylhydrazine-1-¹⁴C hydrochloride from Ba¹⁴CO₃ (starting with 3.00 mmoles of an activity of 3.33 mC/mmole) was 33%. This yield is satisfactory in view of the stability problems encountered in handling hydrazine compounds.

Experimental. Phenylacetic-1-¹⁴C acid. This compound was prepared from benzylmagnesium chloride and ¹⁴CO₂ from 3.00 mmoles of Ba¹⁴CO₃ (activity 3.33 mC/mmole) according to the method described in a previous publication.¹² The yield of crude product, m.p. 70–74°, was 98%.

Phenylacetone-1-¹⁴C. The phenylacetic-1-¹⁴C acid from the foregoing preparation was dissolved in 5 ml of absolute ether in a 25 ml nitrogen-flushed, three-necked flask equipped with a reflux condenser. Methyllithium⁹ (17 ml of a 0.48 M solution in ether = 8 mmoles) was then added cautiously through the condenser and the reaction mixture was refluxed for half an hour. The flask was cooled in an ice-bath, 5.0 ml of water added, and the ethereal layer separated and washed with two 2 ml portions of water. Evaporation of the ether gave crude phenylacetone-1-¹⁴C, which was used directly in the following step.

Phenylacetone-1-¹⁴C γ -glutamylhydrazone. To the crude phenylacetone-1-¹⁴C was added a solution of 0.50 g of γ -glutamylhydrazide¹⁰ in 6.0 ml of water and the reaction mixture was shaken at room temperature for 15 h.

2-Phenylisopropyl-1-¹⁴C- γ -glutamylhydrazine. The dispersion from the foregoing preparation was transferred to a Parr low-pressure hydrogenation bottle with 25 ml of methanol (analytical grade). Adam's catalyst (0.10 g) was added and the mixture hydrogenated at room temperature and 60 p.s.i. for 4 h. The platinum oxide was filtered off and the filtrum washed with three 5 ml portions of methanol. The filter was freed from solvent by evaporation *in vacuo* in a rotating flask evaporator at 40°.

2-Phenylisopropylhydrazine-1-¹⁴C hydrochloride. The hydrazide was dissolved in 4.0 ml of concentrated hydrochloric acid and the solution kept at 100° for 20 min. After cooling, water (6.0 ml) was added and some oily by-

products were taken up in ether. The aqueous layer was chilled in an ice-bath and made alkaline by careful addition of 50% aqueous potassium hydroxide. The hydrazine was taken up in ether (10 + 5 + 5 ml) and immediately precipitated as the hydrochloride by the addition of an excess of hydrogen chloride dissolved in ether. The initially oily hydrochloride crystallized upon seeding with authentic 2-phenylisopropylhydrazine hydrochloride, and the ether was decanted off and the hydrochloride recrystallized from acetonitrile. Filtration yielded 2-phenylisopropylhydrazine-1-¹⁴C hydrochloride (0.175 g, 33% over-all yield), m.p. 113–116°, identical with an authentic inactive specimen according to the mixed m.p. The crystals were dried over concentrated sulphuric acid *in vacuo* for 2 h and then stored in a sealed, nitrogen-filled ampoule at –20°. It is essential that this preparation is made as fast as possible and especially that the handling time of the free hydrazine in solution is kept at a minimum.

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1. Pletscher, A., Gey, K. F. and Zeller, P. *Progr. Drug Res.* **2** (1960) 417.
2. *Ann. N.Y. Acad. Sci.* **107** (1963) 809 f.
3. Zeller, P., Gutmann, H., Hegedüs, B., Kaiser, A., Langemann, A. and Müller, M. *Experientia* **19** (1963) 129.
4. Bollag, W. and Grunberg, E. *Experientia* **19** (1963) 130.
5. Rutishauser, A. and Bollag, W. *Experientia* **19** (1963) 131.
6. Berneis, K., Kofler, M., Bollag, W., Kaiser, A. and Langemann, A. *Experientia* **19** (1963) 132.
7. Ebersson, L. and Persson, K. *J. Med. Pharm. Chem.* **5** (1962) 738.
8. Murray, A. and Williams, D. L. *Organic Syntheses with Isotopes*, Part I, Interscience Publishers, New York 1958, p. 512.
9. Tegnér, C. *Acta Chem. Scand.* **6** (1952) 782.
10. Biel, J. H., Drukker, A. E., Mitchell, T. F., Sprengler, E. P., Nuhfer, P. A., Conway, A. G. and Horita, A. *J. Am. Chem. Soc.* **81** (1959) 2805.
11. Edward, J. T., Hutchison, H. P. and Meacock, S. C. R. *J. Chem. Soc.* **1955** 2520.
12. Ebersson, L. *Acta Chem. Scand.* **16** (1962) 781.

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