THE SYNTHESIS OF ^{14}C-DIETHYLAMINE AND LYSERGIC ACID DIETHYLAMIDE.

R.D. Barnes.
Department of Biochemistry,
St.Mary's Hospital Medical School,
London, W.2 1PG. U.K.
Received on December 12th 1973.

SUMMARY

The preparation of lysergic acid [^{14}C$_1$]-diethylamide is described. [^{14}C$_1$]-Diethylamine was synthesised in three steps; sodium [^{14}C$_1$]-acetate was treated with thionyl chloride giving [^{14}C$_1$]-acetyl chloride which was reacted with ethylamine to give N-ethyl-[^{14}C$_1$]-acetamide. Reduction of this amide with lithium aluminium hydride gave [^{14}C$_1$]-diethylamine which on treatment with a lysergic acid-imidazole complex gave the required product.

Introduction

As part of a study into the metabolism of lysergic acid diethylamide (LSD), the synthesis of carbon-14 labelled LSD has been investigated. Incorporation of carbon-14 into the ergoline ring is not feasible and the two possibilities for labelling are the diethylamide side chain attached at C_8 and the methyl group attached at N_6. Carbon-14-LSD labelled in the diethylamide side chain has already been prepared[1] and in metabolic studies[2] only 4% of the carbon-14 was lost as [^{14}C]-carbon dioxide. Thus LSD was prepared containing carbon-14 in the diethylamide side chain.

The method used by Stoll et al.[1] for the synthesis of [^{14}C$_1$]-diethylamine involved

catalytic hydrogenation of [^{14}C]-acetonitrile to give a mixture of diethylamine and ethylamine.

$$CH_3{}^{14}CN \longrightarrow (CH_3{}^{14}CH_2)_2NH + CH_3{}^{14}CH_2NH_2 + NH_3$$

The two amines were separated by counter current distrubution and the yield of [$^{14}C_1$]-diethylamine was increased by reacting the ethylamine with ammonia and acetaldehyde.

$$CH_3{}^{14}CH_2NH_2 + CH_3CHO + NH_3 \longrightarrow (CH_3{}^{14}CH_2)_2NH + CH_3CH_2NH_2$$

In view of the difficulties in purification and the necessity of synthesising the [^{14}C]-acetonitrile, the following scheme has been used in the present preparation:

$$CH_3{}^{14}COONa \xrightarrow{SOCl_2} CH_3{}^{14}COCl \xrightarrow{EtNH_2} CH_3{}^{14}CONHEt \xrightarrow{LiAlH_4} CH_3{}^{14}CH_2NHEt$$

No purification of the intermediates was necessary and the final solution of [$^{14}C_1$]-diethylamine in ether was suitable for the subsequent formation of LSD.

The incorporation of the [$^{14}C_1$]-diethylamine into LSD was carried out by Stoll et al.[1] by reaction with isolysergic acid azide. A more convenient method is that of Cerny and Semonsky[3] which uses a lysergic acid-imidazole complex, formed in situ from lysergic acid and N,N'-carbonyl-diimidazole. This reaction is reported to give excellent yields when equimolar ratios of lysergic acid and amine are used.

Experimental

Chemicals

Sodium [$^{14}C_1$]-acetate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. (+)-Lysergic acid (Koch-Light Laboratories, Colnbrook, Bucks., U.K.) was purified prior to use by solution in warm 2N-ammonia followed by precipitation

with 2N-acetic acid[4]. N,N'-Carbonyldiimidazole was purchased from Fluka A. G., Buchs S. G., Switzerland.

Chromatography

Thin layer chromatography was carried out on purchased aluminium roll, Silica Gel F_{254} (E. Merck A. G., Darmstadt, Germany). The R_F values for LSD, iso-LSD and lysergic acid in the solvent system, chloroform : methanol (3 : 2, v/v) are 0.74, 0.45 and 0.06 respectively. The spots were detected by fluorescence under UV light (254 nm) and by spraying with 1% N,N-dimethylaminobenzaldehyde in ethanol : aq. HCl soln. (sp. gr. 1.18) (1 :1, v/v).

Gas liquid chromatography was carried out on a Hewlett Packard Chromatograph (F. and M. Scientific 402 High Efficiency Gas Chromatograph) fitted with a flame ionisation detector. A 5 ft glass column (3 mm I.D.) packed with P.E.G. 6000 (10%) and KOH (2%) on Chromosorb G (80-100 mesh) was used. Operating conditions: for N-ethylacetamide column temperature 150°, N_2 flow rate 150 ml/min, retention time 3.3 min; for diethylamine, column temperature 75°, N_2 flow rate 30 ml/min, retention time, 1.5 min.

Radiochemical Techniques

Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer (models 3214 and 3320) using a dioxan-based scintillator fluid[5]. Scans of thin-layer chromatograms were made with a Packard radiochromatogram scanner (model 7200). Also 0.5 cm wide sections of the absorbent were scraped into scintillation vials, scintillator fluid added and the radioactivity counted in the spectrometer.

Synthesis a) [$^{14}C_1$]-Diethylamine

Thionyl chloride (160 mg) in dry ether (1 ml) was added to sodium [$^{14}C_1$]-acetate (7.25 mg; 5 mCi) dried over P_2O_5 under vacuum. The mixture was stirred for 20 min at room temperature and then similarly dried sodium acetate (63 mg) was added. After stirring for a further 20 min the solvent was removed and the solid washed with dry ether (3x1 ml). The pooled solution was stirred, ethylamine (300 mg) in dry ether (2 ml) added slowly drop by drop and after cooling to 0° the precipiate was filtered off. The filtrate was then evaporated at 0° by a stream of dry nitrogen; the residue was taken up in dry ether (2 ml) and analysis showed a chemical yield of N-ethyl-acetamide of 55% (g.l.c.) and a radiochemical yield of 48% (liquid scintillation counting). The solution of N-ethyl-[$^{14}C_1$]-acetamide in dry ether (1.5 ml) was added drop by drop to a stirred suspension of $LiAlH_4$ (160 mg) in dry ether (4 ml) at 20° and the resulting mixture was stirred at room temperature for 16 h. The suspension was then boiled under reflux for 3 h and after cooling to 0°, water was added drop by drop to decompose the excess $LiAlH_4$. A violent reaction occurred with loss of some radioactivity from the top of the condenser. When the reaction stopped, the mixture was transferred to a stoppered test tube, washed in with 10% NaOH, and saturated with NaCl. The mixture was then shaken vigorously and the ether layer removed after it had separated. The aqueous phase was re-extracted with ether (4 x 2 ml) and the pooled extracts dried over Na_2SO_4. Analysis gave a chemical yield of diethylamine of 31% (g.l.c.) and a radiochemical yield of 32% (liquid scintillation counting) with respect to sodium acetate.

Synthesis b) Lysergic acid[$^{14}C_1$]-diethylamide

(+)-Lysergic acid (98 mg) dried over P_2O_5 under vacuum was suspended in dry DMF (8 ml) and N,N'-carbonyldiimidazole (252 mg) was added. After stirring the solution for 30 min in the dark at room temperature, [$^{14}C_1$]-diethylamine (19 mg, 1.6 mCi) in dry ether (9 ml) was added drop by drop. The extent of reaction was determined

by t.l.c. and a further quantity of diethylamine (7 mg) was added to increase the yield of LSD. After a further 2½h stirring, the solvent was removed by evaporation under reduced pressure, and the residue dissolved in 2% (+)-tartaric acid (30 ml). This acidic solution (pH 4) was extracted with ether : ethanol (9:1, v/v) (4 x 25 ml), the pH adjusted to 9 with 10% NaOH and re-extracted with ether : ethanol (9:1, v/v) (6 x 25 ml). T.l.c. showed that almost all the LSD and iso-LSD was present in the basic extract which was then evaporated to dryness under reduced pressure, taken up in the minimum amount of methanol and streaked onto 4, 20 x 20 cm Silica Gel H plates (0.75 mm), pre-eluted twice with methanol. After elution with chloroform : methanol (3:2, v/v) the two fluorescent bands were scraped off, eluted with methanol and assayed by t.l.c. and radiochromatogram scanning. This showed traces of iso-LSD in the LSD and also some LSD in the iso-LSD.

The [^{14}C]-LSD was purified by crystallisation from diisopropyl ether after exhaustive drying over P_2O_5 under vacuum. The yield was 29 mg (26%), specific activity 13.59 μCi/mg). A second crop of [^{14}C]-LSD of lower specific activity was obtained by the addition of LSD (25 mg) to the mother liquor of the above preparation, followed by recrystallisation (yield 25 mg, specific activity 2.78 μCi/mg).

Purity of [^{14}C]-LSD

The mass spectrum (Varian MAT CH5) and i.r. spectrum (Perkin Elmer Infracord 137) are identical with a reference compound (Sandoz Ltd., Basle, Switzerland) and thin-layer chromatography in five systems [A, chloroform : methanol (3:2, v/v); B, acetone : chloroform (4:1, v/v); C, methanol : dioxan (3:2, v/v); D, chloroform : methanol : acetic acid (60:40:1, v/v); E, acetone : chloroform : aq. NH_3 soln. (sp. gr. 0.88) (50:40:1.5, v/v)] gave only one spot. Radiochromatogram scanning and counting sections in 3 different solvent systems (A, B and C) gave a radiochemical purity of ~97%.

Discussion

This method of lysergic acid[$^{14}C_1$]-diethylamide synthesis has advantages over the method of Stoll et al.[1] The two counter current distribution procedures and the column chromatographic purification of LSD have been eliminated with a considerable saving in time. The preparation is also carried out on a much smaller scale, enabling a higher specific activity in the final product.

The yield of N-ethylacetamide can be increased if acetic acid is used as starting material. In this case, acetyl chloride is obtained in a similar yield (~90%) but the reaction with ethylamine proceeds more smoothly to give a higher yield of N-ethylacetamide (85% as compared with 55-65%)[6]. The yield on the reduction stage is poor (~60%) but this is probably due to losses during destruction of the excess lithium aluminium hydride, accentuated by the small scale of the preparation. Again the yield on the final stage, preparation of lysergic acid[$^{14}C_1$]-diethylamide, is low. This is due to formation of iso-LSD and losses in chromatography and crystallisation.

Acknowledgement

The author is grateful to Professor R. T. Williams for his interest in this work which was supported by the Medical Research Council.

References

1. Stoll, A., Rutschmann, J. and Hofmann, A. Helv. Chim. Act 37, 820 (1954).
2. Boyd, E.S. Arch. int. pharmacodyn. 120, 292 (1959).
3. Cerny, A. and Semonsky, M. Collection Czechoslav. Chem. Commun. 27, 1585 (1962).
4. Smith, S. andTimmis, G.M. J. Chem. Soc. 1440 (1936).
5. Bray, S.A. Anal. Biochem. 1, 279 (1960).
6. Barnes, R.D. unpublished observations.