791. *The Synthesis of* ^{14}C-*Labelled Diethylcarbamazine, 1-Diethylcarbamyl-4-methylpiperazine* (" *Hetrazan* ").

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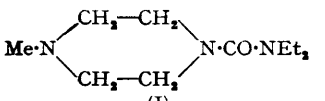
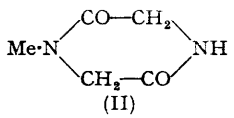
A method has been developed for the conversion of iminodiacetic acid into 1-methylpiperazine in good yield. The use of radioactive starting material has led to the preparation of ^{14}C-labelled 1-diethylcarbamyl-4-methylpiperazine.

The introduction of 1-diethylcarbamyl-4-methylpiperazine (" Hetrazan ") (I) (Kushner, Brancone, Hewitt, McEwen, SubbaRow, Stewart, Turner, and Denton, *J. Org. Chem.*, 1948, **13**, 144) has been a major advance in the treatment of filariasis (see, *inter al.*, Kenney and Hewitt, *Amer. J. Trop. Med.*, 1949, **29**, 89). Little, however, is known of the fate of the drug in the body, and the synthesis of labelled material was therefore undertaken.

1-Diethylcarbamyl-4-methylpiperazine labelled in the methyl group has already been prepared (Arnstein, unpublished work), but in view of the possibility of demethylation in the animal body it was desirable also to have available material labelled in the piperazine ring. The most favourable approach appeared to be *via* 1-methylpiperazine and diethylcarbamyl chloride (Kushner *et al.*, *loc. cit.*). Published methods for the preparation of 1-alkylpiperazines were unpromising, so we turned to the piperazinediones. In preliminary experiments glycine was converted *via* the chloroacetyl derivative into sarcosylglycine (Levene, Simms, and Pfaltz, *J. Biol. Chem.*, 1924, **61**, 445), which was readily cyclised to 1-methylpiperazine-2 : 5-dione (II) in boiling ethylene glycol. A similar method has been



(I)



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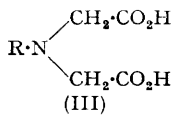

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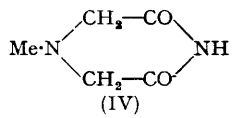


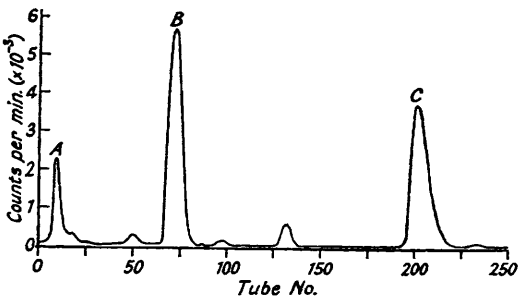
(IV)

used by Sannié (*Bull. Soc. chim.*, 1942, **9**, 487) to convert glycine into piperazine-2 : 5-dione. It may be noted that cyclisation involving a secondary amino-group appears to be more difficult than for a primary one : thus Maurer and Schiedt (*Z. physiol. Chem.*, 1932, **206**, 125) record that the ethyl ester of sarcosylglycine, in contrast to that of glycylglycine, shows no tendency to cyclise spontaneously at room temperature. Reduction of 1-methylpiperazine-2 : 5-dione with lithium aluminium hydride afforded 1-methylpiperazine.

The availability of [$\alpha\alpha'$-$^{14}C_2$]iminodiacetic acid (see below) enabled a different approach to be studied. In preliminary experiments using unlabelled material, iminodiacetic acid (III; R = H) was conveniently prepared by hydrogenolysis of benzyliminodiacetic acid (III; R = Ph·CH$_2$) obtained by treatment of benzylamine with chloroacetic acid in the presence of alkali [cleavage of substituted benzylamines by hydrogen in the presence of palladium is well known (Birkofer, *Ber.*, 1942, **75**, 429)]. Iminodiacetic acid with formic acid and formaldehyde (Childs, Goldsworthy, Harding, King, Nineham, Norris, Plant, Selton, and Tompsett, *J.*, 1948, 2174) afforded methyliminodiacetic acid (III; R = Me) in 95% yield. Fusion with urea then yielded 87% of 1-methylpiperazine-3 : 5-dione (IV). This has been prepared less conveniently by Dubsky (*Ber.*, 1916, **49**, 1039) by sublimation of the diamide obtained by ammonolysis of the dimethyl ester. The use of urea for the direct conversion of dicarboxylic acids into imides appears to have received little attention although Guareschi (*Bull. Soc. chim.*, 1888, **49**, 299) has prepared camphoric imide in this way. The analogous conversion of monocarboxylic acids into amides has been studied by Cherbuliez and Landolt (*Helv. Chim. Acta*, 1946, **29**, 1438). Reduction of 1-methylpiperazine-3 : 5-dione with lithium aluminium hydride afforded 1-methylpiperazine in 77% yield, an overall yield of 64% from iminodiacetic acid. The above reactions were readily adaptable to the millimole scale requisite for the synthesis of labelled diethylcarbamazine.

In the preparation of [α-^{14}C]glycine by treatment of [α-^{14}C]bromoacetic acid with aqueous ammonia a number of by-products are formed including iminodiacetic acid, tri(carboxymethyl)amine, and glycollic acid (cf. Heintz, *Annalen*, 1865, **136**, 213). The mother-

liquors from two such preparations were chromatographed on a column of " Dowex-50," whereby an excellent separation was obtained of the residual glycine and of the required iminodiacetic acid, isolated as their hydrochlorides (see Fig.). No attempt was made to isolate the other components. Treatment of iminodiacetic acid hydrochloride with pyridine in aqueous alcohol yielded free iminodiacetic acid, the identity of which was confirmed by dilution analysis. Methylation, followed by fusion with urea and reduction



as outlined above, afforded 1-methyl-[2 : 6-$^{14}C_2$]piperazine hydrochloride of high specific activity. Treatment of this with diethylcarbamyl chloride and triethylamine in chloroform at room temperature, a modification of the method of Kushner *et al.* (*loc. cit.*), afforded 1-diethylcarbamyl-4-methyl-[3 : 5-$^{14}C_2$]piperazine (I), isolated as the citrate in 90% yield. The identity of the material was confirmed by paper chromatography and by isotopic dilution with an authentic specimen.

Experimental

N-*Benzyliminodiacetic Acid* (III; R = Ph·CH_2).—*N*-Benzyliminodiacetic acid was prepared by an adaptation of the method for methyliminodiacetic acid (*Org. Synth.*, Coll. Vol. II, 1943, p. 397), an equivalent amount of benzylamine being employed in place of the methylamine. The reaction time was increased to 14 hr. and the product isolated as the barium salt (234 g.). After addition of the theoretical volume of 5N-sulphuric acid, filtration, and extraction of the barium sulphate with hot water, the combined extracts were concentrated under reduced pressure. On recrystallisation from water the acid formed colourless needles (111 g., 50%), m. p. 204° (decomp.) (Found: C, 59·0; H, 5·8; N, 6·0. Calc. for $C_{11}H_{13}O_4N$: C, 59·2; H, 5·9; N, 6·3%). Dubsky (*Ber.*, 1921, **54**, 2661) records m. p. 197—198° (decomp.), and Ziemlak, Bullock, Bersworth, and Martell (*J. Org. Chem.*, 1950, **15**, 255) record m. p. 214° (decomp.).

N-*Methyliminodiacetic Acid* (III; R = Me).—Benzyliminodiacetic acid (20 g.), 10% acetic acid (300 c.c.), and 5% palladised charcoal (3 g.) were shaken in an atmosphere of hydrogen until absorption was complete (3 hr). The solution was filtered and concentrated to small volume under reduced pressure, and absolute ethanol was added. The iminodiacetic acid (10·54 g., 88%) formed colourless prisms, m. p. 232° (decomp.). A specimen, crystallised from water, had m. p. 235° (decomp.) (Found: C, 35·9; H, 5·5; N, 10·6. Calc. for $C_4H_7O_4N$: C, 36·1; H, 5·3; N, 10·5%). The m. p. has been recorded as 225° (Polstorff and Meyer, *Ber.*, 1912, **45**, 1905), 235—236° (Bailey and Read, *J. Amer. Chem. Soc.*, 1914, **36**, 1759), and 245° (Jongkees, *Rec. Trav. chim.*, 1908, **27**, 294).

Iminodiacetic acid (1·33 g.), 95% formic acid (1·5 g.), and 40% formaldehyde (1·2 g.) were heated under reflux on the steam-bath for 7 hr., the solvent removed under reduced pressure, and the residue triturated with absolute ethanol (15 c.c.). *N*-Methyliminodiacetic acid (1·39 g., 95%) had m. p. 215—216° (decomp.), undepressed on admixture with an authentic specimen (*Org. Synth.*, Coll. Vol. II, 1943, p. 397). Childs *et al.* (*loc. cit.*) record 83% yield for a similar method.

1-*Methylpiperazine*-3 : 5-*dione* (IV).—A finely crushed mixture of methyliminodiacetic acid (2·94 g.) and urea (1·26 g.) was heated in an open test-tube fitted with a side-arm. The bath-temperature was raised until frothing commenced (160°). When gassing was largely complete the temperature was raised to 170° during 1 hr. Sublimation (cold finger) at 170—190° (bath)/15 mm. yielded 1-methylpiperazine-3 : 5-dione (2·22 g., 87%) as a colourless crystalline solid, m. p. 98—100·5°. Recrystallisation from *n*-propanol yielded colourless leaflets m p

103—104° (Found: C, 47·1; H, 6·0; N, 21·9. Calc. for $C_5H_8O_2N_2$: C, 46·9; H, 6·3; N, 21·9%). Dubsky (*Ber.*, 1916, **49**, 1039) records m. p. 105—106° for this compound prepared by sublimation of *N*-methyliminodiacetamide.

1-*Methylpiperazine*-2 : 5-*dione* (II).—A mixture of sarcosylglycine (3·0 g.) and ethylene glycol (18 c.c.) was boiled under reflux for 30 min. After removal of the solvent at 0·5 mm., the residue was recrystallised from *iso*propanol, to give 1-*methylpiperazine*-2 : 5-*dione* (1·92 g., 73%) in nacreous leaflets, m. p. 141—143° (Found: C, 46·7; H, 6·4; N, 21·9. $C_5H_8O_2N_2$ requires C, 46·9; H, 6·3; N, 21·9%).

1-*Methylpiperazine*.—To a vigorously stirred suspension of lithium aluminium hydride (20 g.) in dry tetrahydrofuran (400 c.c.) was added slowly a solution of 1-methylpiperazine-3 : 5-dione (20 g.) in the same solvent (400 c.c.). The mixture was boiled under reflux for 4 hr., cooled in ice, and diluted with ether (400 c.c.), and water (25 c.c.) was added dropwise. The inorganic material was filtered off and extracted with ether in a Soxhlet apparatus. The combined extracts were concentrated to small bulk on the steam-bath in a flask containing acetic acid (60 c.c.). Concentrated hydrochloric acid (50 c.c.) was then added and the solvent removed under reduced pressure. The last traces were removed by the addition of ethanol (50 c.c.) and evaporation under reduced pressure. Recrystallisation from ethanol yielded 1-methylpiperazine dihydrochloride monohydrate (23·2 g., 77%). An air-dried sample had m. p. 84—86°, but a specimen dried *in vacuo* over phosphoric oxide, first at room temperature and then at 100°, had m. p. 242—243° (Found: C, 34·6; H, 8·4; N, 16·0. Calc. for $C_5H_{12}N_2,2HCl$: C, 34·7; H, 8·2; N, 16·2%). The dipicrate separated from dimethylformamide–ethanol in yellow needles, m. p. 265° (decomp.) (Found: C, 36·5; H, 3·3; N, 19·9. Calc. for $C_5H_{12}N_2,2C_6H_3O_7N_3$: C, 36·6; H, 3·3; N, 20·1%). The m. p. of the dihydrochloride monohydrate has been reported as 82·5—83° (Stewart, Turner, Denton, Kushner, Brancone, McEwen, Hewitt, and SubbaRow, *J. Org. Chem.*, 1948, **13**, 136), 90—91° (Prelog and Štěpán, *Chem. Zentr.*, 1935, II, 2817), and 110° (Baltzly, Buck, Lorz, and Schön, *J. Amer. Chem. Soc.*, 1944, **66**, 263). Prelog and Štěpán (*loc. cit.*) report m. p. 242—243° for the anhydrous material and m. p. 272° (decomp.) for the dipicrate.

In a similar experiment in which the 1-methylpiperazine-2 : 5-dione (1·5 g.) was added as a slurry, the mixture was boiled under reflux for 6 hr. 1-Methylpiperazine was isolated as the picrate (4·25 g., 65%), m. p. 255° (decomp.), raised to 265° on recrystallisation from dimethylformamide–ethanol, and undepressed on admixture with a specimen obtained as above.

Synthesis of Labelled 1-*Diethylcarbamyl*-4-*methylpiperazine*.—Each radioactive compound was assayed after conversion of a diluted sample into barium carbonate. Dry, semi-micro-combustions over copper oxide were performed in duplicate, the carbon dioxide being absorbed directly in hot aqueous barium hydroxide. "Infinitely" thick samples of barium carbonate were counted, a commercial thin-window counter being used. Specific activities (s.a.) were obtained by comparing the counts of the samples with that of a standardised ^{14}C-labelled polymethyl methylacrylate disc of the same size. The specific activities are given as millicuries per millimole (mc/mmole).

Isolation of [$\alpha\alpha'$-$^{14}C_2$]*Iminodiacetic Acid*.—The residues (total activity 25·8 mc; 6·56 mc as iminodiacetic acid) from two [α-^{14}C]glycine preparations, from which excess of ammonia and the bulk of the glycine had been removed, were combined, dissolved in the minimum amount of water, and chromatographed on a column (3·5 × 90 cm.) of "Dowex 50" (1 kg.; 200—400 mesh, previously washed thoroughly with 4N-hydrochloric acid and then with water until the filtrate was neutral). The column was developed with 1·5N-hydrochloric acid. The first 850 c.c. of eluate, which contained an appreciable amount of radioactive material, were not further investigated. Subsequently the eluate was collected automatically in fractions each of 10·6 c.c. A sample (0·01 c.c.) of each fraction was pipetted on to the centre of a 1" watch-glass. After evaporation to dryness at 110°, the samples were counted with an end-window counter. The activities are plotted against tube numbers in the Figure. No attempt was made to identify the minor components. Subsequent elution with 2·5N-hydrochloric acid afforded only ammonium chloride. The contents of the tubes with activities comprising the peaks *A*, *B*, and *C* in the Figure were combined and evaporated to dryness *in vacuo* at *ca.* 50°. Samples of each were chromatographed on Whatman No. 1 paper with phenol–water–ammonia, each spot having been neutralised with ammonia before the chromatogram was run. *A* appeared to be a mixture of two components and was not further studied. The R_F values of *B* and *C* were identical with those of authentic iminodiacetic acid and glycine respectively (autoradiograph and ninhydrin). The identity of each was confirmed by isotopic dilution analysis. The total yield of iminodiacetic acid hydrochloride was 204·9 mg. (5·90 mc; s.a. 4·88).

N-*Methyl*[$\alpha\alpha'$-$^{14}C_2$]*iminodiacetic Acid*.—To a filtered solution of iminodiacetic acid hydrochloride (204·0 mg.; 5·88 mc) in water (*ca.* 1·5 c.c.) was added absolute ethanol (10 c.c.), followed by pyridine (dropwise) until precipitation appeared complete. After 2 hr. at 0° the iminodiacetic acid (112·3 mg.; 4·21 mc; s.a. 4·98) was filtered off. The addition of two portions of inactive carrier iminodiacetic acid (total, 72·5 mg.) to the mother-liquors followed by concentration of the solution and precipitation with absolute ethanol afforded two further crops (65·9, 29·6 mg.). The total radiochemical yield was 5·49 mc (93%). A portion of the [$\alpha\alpha'$-$^{14}C_2$]-iminodiacetic acid (144·4 mg.; 3·19 mc; s.a. 2·94) was methylated with formic acid and formaldehyde as described above (yield: 146·4 mg., 92%). A paper chromatogram employing phenol–water–ammonia showed a single spot of R_F 0·52 (autoradiograph). No trace of iminodiacetic acid (R_F 0·22) could be detected with ninhydrin.

1-*Methyl*[2 : 6-$^{14}C_2$]*piperazine*.—The methyliminodiacetic acid (146·4 mg.; 2·93 mc) was dissolved in water, filtered into the tube of a small sublimation apparatus, evaporated to dryness in a current of air, and heated with urea (62 mg.) as described above. The white sublimate was dissolved in ethanol, the solution concentrated to small bulk, and the 1-methyl-[2 : 6-$^{14}C_2$]-piperazine-3 : 5-dione (76·8 mg.; 1·76 mc; s.a. 2·93; 60%) filtered off.

To a vigorously stirred solution of lithium aluminium hydride (230 mg.) in dry ether (25 c.c.) was added a solution of 1-methyl-[2 : 6-$^{14}C_2$]piperazine-3 : 5-dione (76·8 mg.; 1·76 mc) diluted with inactive material (96 mg.) in dry tetrahydrofuran (5 c.c.). The mixture was boiled under reflux for 4 hr. and then cooled, and water (5 c.c.) was added dropwise. The mixture was made acid with 2N-hydrochloric acid, stirred for 1 hr., and the ether and tetrahydrofuran were largely removed by distillation. The residue was then made strongly alkaline with aqueous sodium hydroxide and distilled in steam into a receiver containing dilute hydrochloric acid (0·01 mole). Distillation was continued until samples showed no activity. The distillate (1·25 l.) was evaporated to dryness under reduced pressure. The 1-methyl-[2 : 6-$^{14}C_2$]piperazine dihydrochloride monohydrate (188·3 mg.; 1·26 mc; s.a. 1·28; 72%), which separated from absolute ethanol containing a few drops of concentrated hydrochloric acid in colourless plates, was filtered off and dried *in vacuo*.

1-*Diethylcarbamyl*-4-*methyl*[3 : 5-$^{14}C_2$]*piperazine*.—To 1-methyl[2 : 6-$^{14}C_2$]piperazine dihydrochloride monohydrate (97·6 mg.; 0·65 mc; s.a. 1·28) was added a solution of triethylamine (0·22 c.c.) in dry chloroform (4 c.c.), followed by a solution of diethylcarbamyl chloride (76·5 mg.) in dry chloroform (1 c.c.), and the mixture was kept overnight at room temperature. After the removal of most of the chloroform in a stream of air, dry ether (15 c.c.) was added, and the triethylamine hydrochloride filtered off and washed with ether. The filtrate and washings were concentrated to 5 c.c. and treated with a solution of citric acid (115 mg.) in ether (10 c.c.). The 1-diethylcarbamyl-4-methyl[3 : 5-$^{14}C_2$]piperazine dihydrogen citrate separated as an oil which readily solidified when scratched and was filtered off, washed well with ether, and dried *in vacuo* (yield: 179·9 mg.; 0·58 mc; s.a. 1·27; 90%). A paper chromatogram in pyridine–pentyl alcohol–water revealed only one spot (autoradiograph) of R_F 0·75. Under the same conditions 1-methylpiperazine showed R_F 0·18. The purity was further confirmed by isotopic dilution analysis with authentic 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate.

The authors thank Dr. J. Walker for suggesting this research, and for advice, Dr. J. R. Catch for his helpful interest, and Mr. W. A. L. Marshment for technical assistance. One of them (A. M. D.) is an Officer of the Commonwealth Scientific and Industrial Research Organisation (Australia).

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[*Received, July 2nd*, 1953.]