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Structures of the Major Metabolites of Meprobamate

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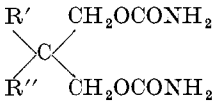
Meprobamate (I) was found by Berger[1] and others[2] to be excreted in the urine of animals and humans in an unchanged form, and as a glucosyluronide conjugate. More recently, Walkenstein *et al.*[3] reported the isolation of a second metabolic product, 2-hydroxymethyl-2-propyl-1,3-propanediol dicarbamate (II), from urine of animals receiving this drug. As part of our study of the metabolism of meprobamate,[4] we had occasion to synthesize the proposed metabolite (II), and to utilize it in paper chromatographic studies of urine derived from meprobamate-fed animals and humans. A slight but consistent divergence between the R_f values for (II) and the actual urinary end-product (0·78 and 0·75 respectively) raised the suspicion that the structure proposed by Walkenstein *et al.* was incorrect, probably with respect to the position at which hydroxylation of the meprobamate structure was believed to have taken place.



(I); R′ = CH_3, R″ = $CH_3CH_2CH_2$
(II); R′ = $HOCH_2$, R″ = $CH_3CH_2CH_2$
(III); R′ = CH_3, R″ = $CH_3CHOHCH_2$

Fig. 1.

This led us to isolate the metabolite in crystalline form and to compare its physical properties with those of (II). While both substances assayed correctly for a hydroxymeprobamate, the

melting points were distinctly different, (II) melting at 149–152° and the isolate at 95–96°. As shown in Fig. 2, there was also a difference in the R_f values for the pure compound as compared to those for (II) when chromatographed in a butanol-acetic

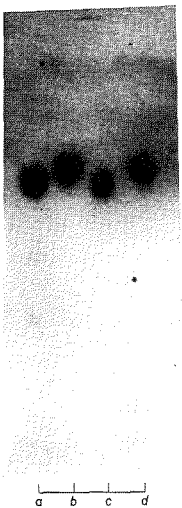


Fig. 2. Paper chromatogram of the major urinary metabolite of meprobamate and 2-hydroxymethyl-2-propyl-1,3-propanediol dicarbamate (II). (*a*) Urinary metabolite, 10 μg; (*b*) compound (II), 10 μg; (*c*) urinary metabolite, 5 μg; (*d*) compound (II), 5 μg.

acid–water system. Infrared spectra comparison (Fig. 3) showed major peak differences at 9·58 μ, the primary alcohol deformation band of (II), and at 9·00 μ, the secondary alcohol deformation band of the metabolite. On the basis of these findings, it became obvious that the two compounds were different and that structure (II), which had been proposed for the metabolite, was incorrect.

To ascertain the true structural nature of the urinary end-product, we compared the triol obtained on its hydrolysis with several other hydroxylated 2-methyl-2-propyl-1,3-propanediols,

obtained by synthesis. These included the hydroxymethyl- (VI), α-hydroxypropyl- (IV) and the β-hydroxypropyl- (V) derivatives. Comparison of these four triols by infrared spectro-

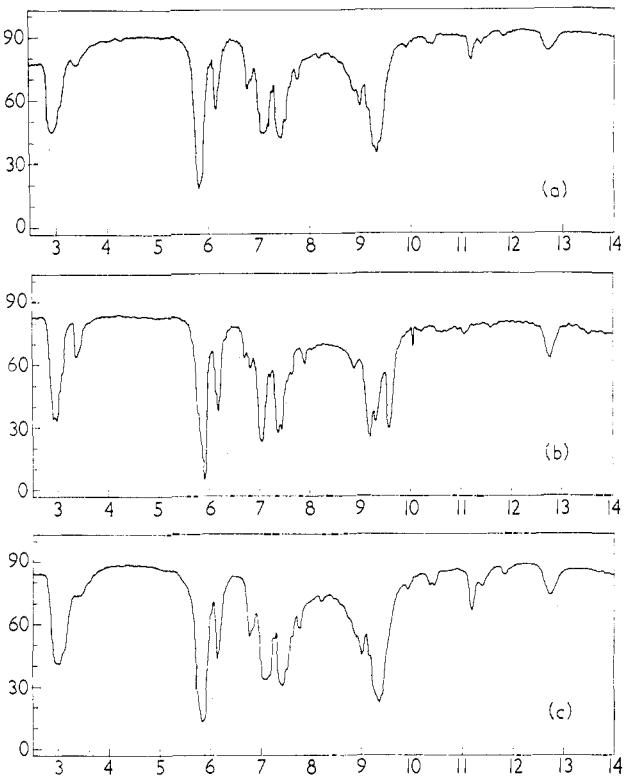


Fig. 3. Infrared spectra of (*a*) the major urinary metabolite of meprobamate, (*b*) 2-hydroxymethyl-2-propyl-1,3-propanediol dicarbamate (II), and (*c*) 2-methyl-2-(β-hydroxypropyl)-1,3-propanediol dicarbamate (III).

scopy, particularly in the 8–10 μ region (Fig. 4), indicated that (V) was identical to the hydrolytic product obtained from the metabolite. This was further confirmed by comparison of their α-naphthyl urethanes. Both derivatives gave the same infrared spectrum and each melted at 95–98° with no depression of melting point on mixing.

As a final link in the chain of structure determination, we

synthesized compound (III) by a route selected to locate unequivocably the position of the hydroxyl group. In this synthesis, shown in Fig. 5, the hydroxyl group of diethyl β-hydroxypropylmethylmalonate was protected by pyranylation (VII) and the ester converted to diol (VIII) using lithium aluminium hydride. Carbamoylation of this diol using phosgene and ammonia gave the tetrahydropyran ether of the desired hydroxydicarbamate (IX) which was converted to (III) by acid hydrolysis. Synthetic

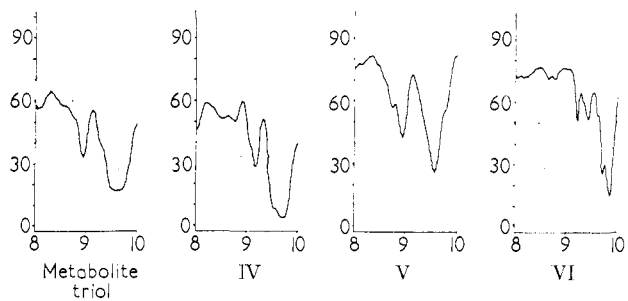


Fig. 4. Infrared spectra in the 8·00–10·00 μ region of several hydroxy derivatives of 2-methyl-2-propyl-1,3-propanediol, where metabolite triol is derived from the major metabolite of meprobamate; (IV) is 2-methyl-2-(α-hydroxypropyl)-1,3-propanediol; (V) is 2-methyl-2-(β-hydroxypropyl)-1,3-propanediol; and (VI) is 1, 1,1-trishydroxybutane.

(III) was found to be identical to the isolated metabolite in R_f value, infrared spectrum (Fig. 3) and melting point, and there was no depression of the latter on mixing the synthetic material with that isolated from animal urine. These findings establish the major unconjugated metabolic product of meprobamate to be 2-methyl-2-β-hydroxypropyl-1,3-propanediol dicarbamate (compound (III), Fig. 1).

Compound (III) proved to be a true detoxification product of meprobamate. In doses as high as 7000 mg/kg given intraperitoneally to mice, it did not produce paralysis of skeletal muscles and did not cause death of any of the animals. Meprobamate given similarly produced paralysis of skeletal muscles, as measured by the loss of the righting reflex, in 50 per cent of animals in doses of 235 mg/kg (PD_{50}) and caused death in 50 per cent of

animals in doses of 800 mg/kg (LD_{50}). The hydroxylated metabolite also possesses a much greater water solubility than meprobamate.

The occurrence of abnormal amounts of reducing substances in the urine of subjects taking excessive amounts of meprobamate

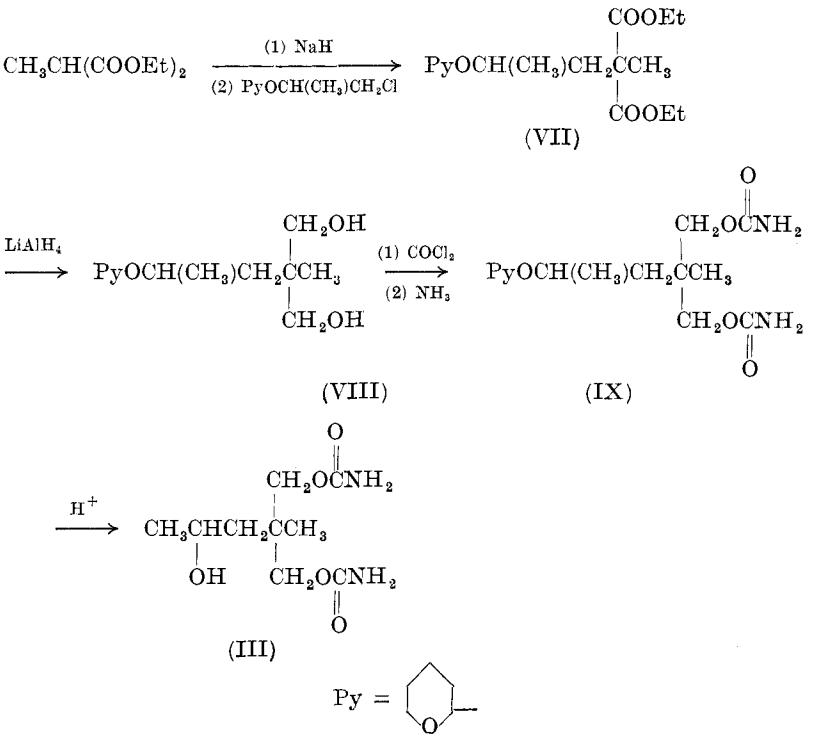


Fig. 5.

suggested the possibility that this compound is excreted partly in the form of a glucuronide.[1] In an attempt to elucidate the nature of this glucosyluronide metabolite, more extensive studies have been conducted using both animal and human subjects. A human subject having a normal 48 h glucuronide excretion equivalent to 430 mg of glucuronic acid excreted 6·4 times this amount over a 48 h period following ingestion of 4 g of meprobamate. Analysis of individual urine specimens using the method

of Deichmann[5] indicated that this abnormal excretion began about 2 h after ingestion of the first portion and continued for at least 24 h after the final dose. On the assumption that the increased glucuronic acid represented a hemiacetal type of conjugate of meprobamate or one of its oxidation products, separation of the glucuronide was affected by means of the insoluble salt formed with basic lead acetate. The free acid obtained by the action of hydrogen sulphide on the lead salt was absorbed on Dowex-1 resin and elution carried out using formic acid. Evaporation of the main fraction at room temperature gave a hygroscopic coloured semi-solid which resisted all attempts to obtain it in a crystalline or solid state, and which darkened progressively on standing. The material was shown by paper chromatography to contain no free meprobamate and to be fairly resistant to acid hydrolysis. Heating of this substance with 4·5 *N* hydrochloric acid at 100° yielded a neutral fraction which after distilling *in vacuo* was readily crystallized. Recrystallization from trichlorethylene gave a product identical in its physical properties and infrared spectrum with meprobamate, and a mixture with an authentic specimen gave no depression in melting point.

The regeneration of unaltered meprobamate from this glucuronide on acid hydrolysis, coupled with the ability of the conjugate to form an insoluble lead salt, the free acid form of which is adsorbable on an anionic exchange resin, characterizes this conjugate as a glucosyluronide of meprobamate. Further studies are in progress to confirm the chemical structure of the compound.

Experimental*

Infrared spectra were determined with a Perkin–Elmer Model 21 Recording Spectrophotometer equipped with sodium chloride optics. Solids were run in potassium bromide pellets and oils between salt windows. Paper chromatography was carried out using Whatman No. 1 paper in the decending technique for 16 h at 27°. Staining of the carbamate groups was effected using a modification of the method of Rydon and Smith.[6]

* All temperatures are uncorrected. Microanalyses by Schwartzkopf Microanalytical Laboratory, Woodside, N.Y.

Isolation of unconjugated metabolite. The major unconjugated metabolite of meprobamate was isolated as follows. A dose of 100 mg/kg of drug was fed to several large dogs and their urine collected under toluene for a 24 h period. The combined urines were then made basic with ammonium hydroxide and continuously extracted with ether for 16 h to remove unchanged meprobamate. A continuous extraction with ethyl acetate for 48 h was then employed to remove the metabolite from the urine. The latter extract was evaporated to dryness under reduced pressure and the residue taken up in a small volume of acetone and filtered to remove urea. Evaporation of the acetone followed by resuspension in acetone and filtration was repeated twice. Before crystallization of the metabolite could be affected it was necessary to purify it further, either by preparative paper chromatography or by column chromatography. The former procedure used butanol–acetic acid–water (4 : 1 : 5) as developing solvent and acetone as eluant, while the latter protocol employed cellulose columns washed with *n*-butanol and the same solvent used as eluant. After the purified chromatographic fractions were treated with charcoal and evaporated, a white crystalline product was obtained from ethyl acetate–petroleum ether, m.p. 95–96°.

Anal. Calcd. for 2-methyl-2-(β-hydroxypropyl)-1,3-propanediol dicarbamate, $C_9H_{18}N_2O_5$: C, 46·16; H, 7·73; N, 11·97. Found: C, 46·08; H, 7·82; N, 12·18.

Preparation of 2-methyl-2-(β-hydroxypropyl)-1,3-propanediol from metabolite. A solution of the metabolite (30 mg) in 1 per cent potassium hydroxide solution (10 ml) was refluxed for 3 h. The hydrolysate was then extracted three times with equal volumes of butanol and the extracts were combined and evaporated. The residue was taken up in methyl propyl ketone, centrifuged, and the supernatant fluid evaporated *in vacuo* to yield a thick colourless syrup of almost pure triol, as determined by infrared spectroscopy.

An α-naphthyl urethane derivative of the triol was prepared by refluxing it with an excess of β-naphthyl isocyanate for 2 h, m.p. 95–98°.

Diethyl methylpropionyl malonate. Diethyl methylmalonate (160 g, 1 mole) was added to sodium hydride (24 g, 1 mole) in toluene (1500 ml). When the evolution of hydrogen had ceased,

the mixture was heated to 100° and then cooled to 25°. Propionyl chloride (101·7 g, 1·1 mole) was then added over 1·5 h while maintaining the temperature at 25–30°. Stirring was continued for 2 h after which the mixture was refluxed for 1·5 h, cooled and then washed successively with water, 10 per cent sodium bicarbonate solution, saturated sodium chloride solution and water. The dried toluene solution was heated under reduced pressure to remove solvent and the residue vacuum distilled. Diethyl methylpropionyl malonate (134 g) was collected at 129–130°/10 mm, n_D^{18} 1·4313.

Anal. Calcd. for $C_{11}H_{18}O_5$: C, 57·38; H, 7·88. Found: C, 58·12; H, 7·59.

2-Methyl-2-(α-hydroxypropyl)-1,3-propanediol (IV). Diethyl methylpropionylmalonate (82 g, 0·30 mole) was added to lithium aluminium hydride (27·4 g, 0·72 mole) dissolved in ether (1500 ml) at such a rate as to maintain rapid reflux. The mixture was stirred and refluxed for an additional 2 h, cooled in an ice bath and treated with 10 ml of ethyl acetate and then with 240 ml of water. The ether layer was decanted from the solids, dried over anhydrous sodium sulphate, concentrated under reduced pressure and the residue vacuum distilled. A total of 20 g of 2-methyl-2-(α-hydroxypropyl)-1,3-propanediol was collected, b.p. 100–104°/0·05 mm, n_D^{29} 1·4725.

Anal. Calcd. for $C_7H_{16}O_3$: C, 56·71; H, 10·88. Found: C, 56·72; H, 10·81.

N,N'-di-(α-naphthyl)-2-methyl-2-(α-hydroxypropyl)-1,3-propanediol dicarbamate was prepared from (IV) and α-naphthyl isocyanate in benzene, m.p. 70–74°.

Preparation of 2-methyl-2-β-hydroxypropyl-1,3-propanediol dicarbamate (III). Diethyl 2-methyl-2-β-(2-tetrahydropyranoxy)propyl malonate (VII). Isomer-free 1-chloro-2-propanol was prepared and assayed by the method of Stewart and Vander Werf[7] and converted to its tetrahydropyran derivative by reaction with dihydropyran in the presence of Dowex-50 resin.[8] Diethyl methylmalonate (174 g, 1 mole) was added to sodium hydride (24 g, 1 mole) in dimethylformamide (1500 ml). When hydrogen evolution had ceased, β-(2-tetrahydropyranoxy)propyl chloride (196 g, 1·1 mole) was added and the mixture heated at 80–90° for 24 h. The mixture was then cooled, diluted with two volumes

of water and extracted with ether. The ether solution was dried (Na_2SO_4 anhyd.), concentrated and the residue vacuum distilled. The product was collected at 83–87°/0·02 mm, n_D^{23} 1·4515, d_4^{20} 1·065.

Anal. Calcd. for $C_{16}H_{28}O_6$: C, 60·73; H, 8·92. Found: C, 61·42; H, 9·02.

2-Methyl-2-(β-2-tetrahydropyranoxypropyl)-1,3-propanediol (VIII) was obtained from (VII) by the method used for the preparation of (V), b.p. 115–18°/0·05 mm, n_D^{24} 1·4759.

Anal. Calcd. for $C_{12}H_{24}O_4$: C, 62·03; H, 10·41. Found: C, 62·22; H, 10·37.

2-Methyl-2-β-(2-tetrahydropyranoxypropyl)-1,3-propanediol dicarbamate (IX). A solution of (VIII) (23·2 g, 0·1 mole) in ether (200 ml) was slowly added with stirring to a solution of phosgene (22 g, 0·22 mole) and pyridine (19 g, 0·24 mole) in ether (300 ml), cooled to 10°. When the addition was complete, the mixture was cooled to −20° and anhydrous ammonia added, the temperature being allowed to rise to 10° during the addition. The solid was filtered off and the filtrate concentrated by distillation under reduced pressure. The oil residue was washed twice with petroleum ether and the residue freed from volatiles by evacuation at steam bath temperature.

Anal. Calcd. for $C_{14}H_{26}N_2O_6$: C, 52·80; H, 8·23; N, 8·80. Found: C, 52·86; H, 7·97; N, 8·69.

2-Methyl-2-(β-hydroxypropyl)-1,3-propanediol dicarbamate (III). A mixture of (IX) (29 g, 0·09 mole), phenylhydrazine hydrochloride (13 g, 0·09 mole), water (250 ml), ethanol (100 ml) and hydrochloric acid (0·5 ml) was placed in a flask and warmed on a steam bath for 1 h. The alcohol was removed under reduced pressure, and the mixture diluted with 400 ml of water and filtered to remove insoluble material. The filtrate was made alkaline with ammonium hydroxide and extracted with four 100 ml portions of ether and four 100 ml portions of chloroform. The water solution was concentrated under reduced pressure and the residue extracted with ethyl acetate. The extract was treated with charcoal, clarified and ether was added until incipient cloudiness occurred. The solution was allowed to stand at room temperature until crystallization took place. The solid was recrystallized from ethyl acetate–ether to a melting point of 93–95°.

Anal. Calcd. for $C_9H_{18}N_2O_5$: C, 46·13; H, 7·74; N, 11·90. Found: C, 46·24; H, 7·68; N, 12·03.

2-Methyl-2-(β-hydroxypropyl)-1,3-propanediol (*V*). Diethyl methyl-β-hydroxypropyl malonate (24 g, 0·125 mole), obtained by acid hydrolysis of (VII), was added to lithium aluminium hydride (9·4 g, 0·25 mole) dissolved in ether (500 ml) at a rate to maintain rapid reflux. The mixture was stirred and refluxed for an additional 2 h, cooled in an ice bath and treated with ethyl acetate and 93 ml of water. The ether layer was decanted, dried and concentrated under reduced pressure, and the oil residue vacuum distilled. The fraction distilling between 102° and 110°/0·02 mm was collected. It weighed 16·4 g (88·7 per cent), n_D^{26} 1·4659.

Anal. Calcd. for $C_7H_{16}O_3$: C, 56·71; H, 10·88. Found: C, 56·59; H, 10·53.

N,N'-Di-α-naphthyl-2-methyl-2-(β-hydroxypropyl)-1,3-propanediol dicarbamate was prepared from (V) and α-naphthyl isocyanate, m.p. 95–98°.

Anal. Calcd. for $C_{28}H_{30}N_2O_5$: C, 71·59; H, 6·23; N, 5·77. Found: C, 73·70; H, 6·02; N, 5·40.

1,1,1-Trishydroxymethylbutane (*VI*). Valeraldehyde (85·7 g, 1 mole) was added over 50 min to a stirred solution of 30 per cent sodium hydroxide (171 g) and 25 per cent formaldehyde (600 ml), the temperature being maintained between 20–25°. The mixture was heated to 55–60° for 4 h, neutralized with formic acid and the solvent was removed under reduced pressure. Then 500 ml of butanol was added and the mixture refluxed for 6 h, allowed to stand for 24 h and filtered. The filtrate was concentrated under reduced pressure, diluted with 500 ml of ether and filtered. The solid was recrystallized from methyl propyl ketone yielding 64 g of 1,1,1-trishydroxymethylbutane, m.p. 100–103°.

Anal. Calcd. for $C_7H_{16}O_3$: C, 56·71; H, 10·88. Found: C, 56·71; H, 10·86.

Isolation of meprobamate glucosyluronide. The combined urine output (3·75 l.) of an adult human male subject, collected over a period of 48 h following ingestion of 4·0 g of meprobamate (during a period of 15 h) was acidified to pH 3·0 with hydrochloric acid and subjected to continuous ether extraction for 40 h. The glucuronic acid content[5] of the extracted urine was 2·6 g, com-

pared to 430 mg found for a 48 h control urine from this same subject. The extracted urine was adjusted to pH 2·6 with acetic acid and treated with an excess of neutral lead acetate. The precipitate which formed was removed by suction filtration. The filtrate was made alkaline while cooling, by adding sufficient ammonium hydroxide to give a pH of 9·0. A slight amount of precipitate which formed was removed and discarded. A solution of basic lead acetate was added until precipitation was complete. The solid was filtered off, washed thoroughly by slurrying with successive portions of water, refiltered, and dried by slurrying with acetone and again filtering. The air-dried crude basic lead acetate precipitate weighed 29·6 g and was found on analysis to contain approximately 60 per cent of the glucuronic acid originally present in the urine.

All attempts to isolate the free glucuronide from this lead salt in a form suitable for characterization were unsuccessful. On evaporation or freeze-drying, in an atmosphere of air or nitrogen, solutions of the glucuronide invariably darkened and eventually deposited a dark insoluble residue as complete dehydration was approached. The following method was found suitable for liberating meprobamate from the lead salt of the glucuronide.

Hydrolysis of meprobamate glucosyluronide. Five grams of the lead precipitate was ground to a fine powder, suspended in 50 ml of water and treated with hydrogen sulphide until lead sulphide precipitation was complete. The sulphide was removed and the filtrate freed from hydrogen sulphide by aeration. The resulting brown-coloured solution was stirred with 10 g of Dowex-1 resin (acid form), filtered and washed with water until the filtrate was neutral. The glucuronide was then eluted from the resin using an excess of formic acid (50 ml, 12 N), giving a total volume including washings of about 125 ml. This solution was concentrated *in vacuo* at 15–20° to a volume of 10 ml. Two further dilutions with water to about 100 ml, followed by evaporation, removed substantially all of the formic acid and left a light brown solution which gave a strong qualitative test for glucuronic acid and liberated ammonia on heating with strong alkali.

The solution was extracted four times with 10 ml portions of ether and the extracts were discarded. The aqueous portion was aerated to remove residual ether and sufficient concentrated

hydrochloric acid added to adjust the solution to 4·5 N. The solution was refluxed for 1 h, the ether soluble fraction extracted, and the extract shaken with dilute sodium carbonate solution to free it of acidic components. Complete evaporation of the solvent under reduced pressure gave approximately 100 mg of a viscous yellow oil which resisted all attempts at crystallization. The oil was distilled at 0·06 mm, and the bulk of the material distilled at 100–120°. On dissolving the oil in hot chloroform and adding petroleum ether, crystallization occurred readily. Further recrystallization from trichlorethylene gave 25 mg of product which melted at 105–106°, and a mixture of this compound with an authentic specimen of meprobamate showed no depression in melting point. The isolated compound was also found to be indistinguishable from meprobamate in its infrared spectrum and behaviour in paper chromatography.

Summary. The structure of the major unconjugated metabolite of meprobamate has been established to be 2-methyl-2-(β-hydroxypropyl)-1,3-propanediol dicarbamate, and not 2-hydroxymethyl-2-propyl-1,3-propanediol dicarbamate as previously reported by others. Evidence is also presented to show that a major conjugated metabolite of meprobamate is a glucosyluronide of unaltered meprobamate.

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References

[1] Berger, F. M. *J. Pharmacol.*, **112**, 413 (1954)
[2] Agranoff, B. W., Bradley, R. M. and Axelrod, J. *Proc. Soc. exp. Biol. N.Y.*, **96**, 261 (1957)
[3] Walkenstein, S. S., Knebel, C. M., MacMullens, J. A. and Seifter, J. *J. Pharmacol.*, **123**, 254 (1958)
[4] Douglas, J. F. and Ludwig, B. J. *Fed. Proc.*, **18**, (1959)
[5] Deichmann, W. *J. Lab. clin. Med.*, **28**, 770 (1943)
[6] Rydon, H. N. and Smith, P. W. G. *Nature, Lond.*, **169**, 922 (1952)
[7] Stewart, C. A. and Vander Werf, C. A. *J. Amer. chem. Soc.*, **76**, 1259 (1954)
[8] Woods, G. F. and Kramer, D. N. *J. Amer. chem. Soc.*, **69**, 2246 (1947)