LABELLING OF A NEW HYPOLIPAEMIC AGENT WITH CARBON-14, PREPARATION OF 1,1-BIS[4-(1-CARBOXY-1-METHYLPROPOXY)PHENYL]CYCLOHEXANE-2-^{14}C

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SUMMARY

1,1-Bis[4-(1-carboxy-1-methylpropoxy)phenyl]cyclohexane (S-8527)(I), a new hypolipaemic agent, was labelled at C-2 with carbon-14 for the use of metabolic studies. The procedure used is illustrated in Fig. 1. Cyclohexane-2-^{14}C which was prepared from potassium cyanide-^{14}C was condensed with phenol to afford 1,1-bis(4-hydroxyphenyl)cyclohexane-2-^{14}C (VI). Condensation of VI with ethyl methyl ketone and chloroform under a strong base (potassium hydroxide) gave S-8527-2-^{14}C (I). A total of 3.11 mCi of pure S-8527-2-^{14}C (I) was obtained, representing 12% radiochemical yield from potassium cyanide-^{14}C. Furthermore, a new radioactive by-product was isolated and elucidated its structure as X (Fig. 3).

Key Words: Aryloxybutyric Acid Derivatives, Hypolipaemic Agent, Carbon-14

INTRODUCTION

A new aryloxybutyric acid derivative, 1,1-bis[4-(1-carboxy-1-methylpropoxy)-phenyl]cyclohexane (S-8527) (I) synthesized in our laboratories[1], was found to have hypolipaemic properties at lower dose levels than clofibrate[2] and less hepatic effects to the effective dose in experimental animals[3-5]. Our interest in the metabolism of the agent has led to the synthesis of the agent specifically labelled at C-2 position of cyclohexane with carbon-14. The labelling position was selected in anticipation of the possible formation of metabolites derived from the biotransformation of the butyric acid moieties.

DISCUSSION

The synthetic pathway devised is shown in Fig. 1.

Among the known procedures[6-8] to prepare cyclohexanone-2-^{14}C from potassium cyanide-^{14}C, we choosed Geiss' method[8] with some modifications. The preparation of 1-hydroxycyclopentanecarbonitrile-^{14}C (II) was accomplished in an aqueous mixture of potassium cyanide-^{14}C and cyclopentanone sodium bisulfite salt[9]. This method was preferable to Geiss' one in which free cyclopentanone was used in stead of the salt. Thus the yield of the cyanohydrin-^{14}C (II) was quantitative. Acetylation of II with acetyl chloride in acetic anhydride followed by reduction with lithium aluminum hydride gave 1-aminomethyl-1-hydroxycyclopentane-^{14}C (IV) in 70% yield. Continuous extraction of the considerablly water-soluble amine (IV) from the reaction mixture by using a Kuscher-Steudel type extractor was found to be effective in this experiment. Ring expansion of IV was achieved with sodium nitrite in 20% acetic acid to afford crude cyclohexanone-2-^{14}C (V), which was rigorously purified by sodium bisulfite

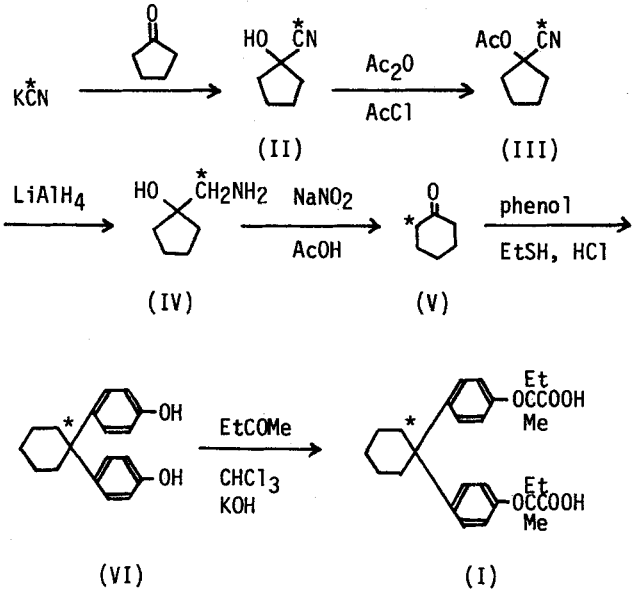

Fig. 1. Scheme for the synthesis of 1,1-bis[4-(1-carboxy-1-methylpropoxy)phenyl]cyclohexane-2-^{14}C

salt formation and recrystallization of the salt since it was observed that the purity of cyclohexanone-^{14}C influenced greatly on the yield of the bisphenol (VI) in the following reaction. The yield of pure cyclohexanone-2-^{14}C (V) was 60% from IV and the purity 99% both radiochemically and chemically.

1,1-Bis(4-hydroxyphenyl)cyclohexane-2-^{14}C (VI) was obtained in 80% yield when cyclohexanone-2-^{14}C and phenol were allowed to react in the presence of hydrochloric acid and ethyl mercaptan[10] as catalysts.

The synthesis of S-8527-^{14}C (I) from VI was accomplished in essentially the same method as described by Nakamura[1]; namely the bisphenol (VI) was reacted with large excesses of chloroform, ethyl methyl ketone and potassium hydroxide to give a mixture of S-8527-^{14}C and various radioactive by-products. In order to obtain a practical method in radiochemical works to isolate S-8527-^{14}C from the by-products we studied their solubility behavior in various bases since S-8527 is a diacid and most of the by-products could be expected to be acidic. Figure 2 shows the scheme of fractionation of the reaction mixture successfully employed. Fractionation of the mixture with 10% sodium carbonate and sodium bicarbonate aqueous solutions gave three fractions (F1, F2 and F3). As would be expected, thin layer chromatographic (TLC) examination of each fraction (see Table 1) revealed that the sodium carbonate-insoluble fraction (F1) contained mainly the starting bisphenol (VI) while F2 consisted of the monoacids (7, 8 and 9) which were already known as the by-products of this reaction[11]. In our case these by-products were isolated and identified by comparing with authentic samples as described in the experimental. TLC of F3 showed single spot virtually identical R_f-value of S-8527 with EtOAc-MeOH-H_2O system, however, with $CHCl_3$-AcOH system it was separated into two spots, and the major one (R_f=0.48) was identical with that of I while the minor one (R_f=0.54, unknown A) was not identical with those of any authentic compounds. Separation of the required S-8527-^{14}C from the mixture was carried out by partial removal of unknown A by column chromatography on silica gel and was completed by fractional crystallization from benzene-*n*-hexane. A total of 3.11 mCi of S-8527-^{14}C (I) was obtained, representing 12% yield from potassium cyanide-^{14}C.

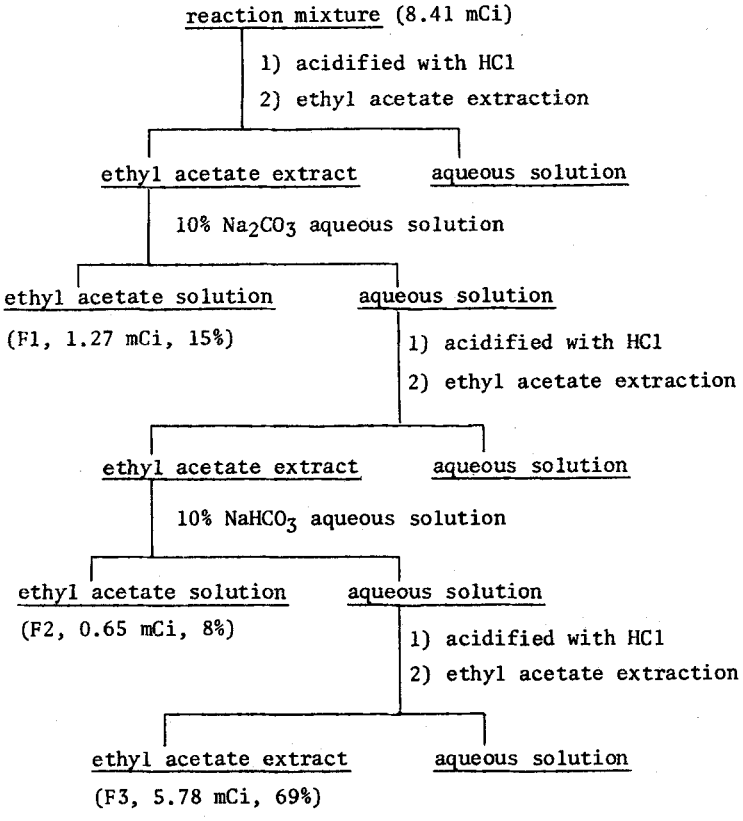

Fig. 2. Scheme for fractionation of reaction mixture of 1,1-bis[4-(1-carboxy-1-methylpropoxy)phenyl]-cyclohexane-2-^{14}C

Table 1. Composition of fractions (F1, F2 and F3) and R_f-value of each product on silica gel-TLC

fraction	compound		R_f-value $CHCl_3$:AcOH =10:1	R_f-value EtOAc:MeOH:H_2O =7:4:0.3
F1	bisphenol	(VI)	0.15	0.63
F2	monoacid	(VII)	0.31	0.55
		(VIII)	0.72	0.48
		(IX)	0.60	0.40
F3	S-8527	(I)	0.48	0.34
	unknown A	(X)	0.54	0.34

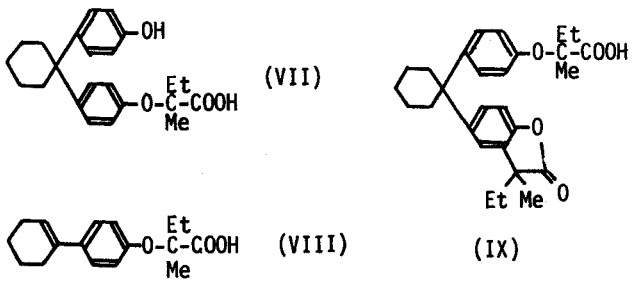

Fig. 3. Structures of Monoacids

Finally, we isolated unknown A from the mother liquid by preparative TLC on silica gel with the solvent system of $CHCl_3$-AcOH and elucidated the structure as follows. Unknown A is a viscous oil and has similar chemical properties to S-8527 (I) as indicated by its solubility in 10% sodium bicarbonate aqueous solution and by its similar mobility on TLC. From the facts unknown A was supposed to be a diacid which may have a structure very close to I. Its IR-spectrum showed absorptions at 3600-2300 (broad) and 1760-1700 (broad and very strong) cm^{-1}; indicating the presence of one ester and two carboxylic acid groups. Its NMR spectrum showed characteristic signals for three primary methyl groups at δ 1.1 (9H, triplet, J=5 Hz), three tertiary methyl groups at δ 1.5 (9H, singlet), eight aromatic protons at δ 6.8 (4H, doublet, J=10 Hz) and 7.1 (4H, doublet, J=10 Hz), and two protons of carboxylic acid at δ 9.0 (broad singlet); bearing a close resemblance to that of S-8527 differing only in the appearance of additional signals for two methyl groups at δ 1.1 and 1.5.

Esterification of unknown A with diazomethane afforded a dimethyl ester. Its mass spectrum showed the molecular ion for $C_{35}H_{48}O_8$ at m/e 596 and prominent peaks as shown in Table 2. The mass spectrum was observed to have considerable identities particularly in the lower mass region below m/e 482 with that of the dimethyl ester of S-8527; the latter indicating the molecular ion for $C_{30}H_{40}O_6$ at m/e 496 and characteristic peaks at m/e 482, 437, 382 and 268 (base peak). The fact suggests that the dimethyl ester of unknown A has a part structure *b* as shown in Fig. 3 and differs only in the molecular weight by 115 ($C_6H_{11}O_2$) from *b*. The residual moiety corresponding to $C_6H_{11}O_2$ could be considered most reasonably to have a structure: C(Me)(Et)COOMe.

Table 2. Prominent Mass Peaks of Dimethyl Ester (XI) of Unknown A

m/e	relative intensity	structure of fragment ion
596	26	molecular ion
573	7	*a*
482	6	*b* + *1*
437	30	*c*
382	35	*d* + *1*
268	100	*e* + *2* (base peak)

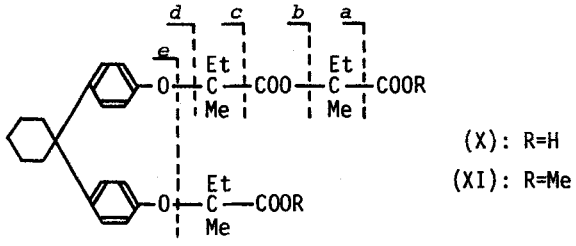

Fig. 3. Structure of Unknown A and Mass Fragmentation of Its Dimethyl Ester

These data all pointed to the probability that unknown A has the structure (X). This conclusion is unequivocally supported by the fact that unknown A is readily hydrolized at the ester group with 10% potassium carbonate aqueous solution to produce S-8527 (I) in good yields.

EXPERIMENTAL

1-Hydroxycyclopentanecarbonitrile-(nitrile-^{14}C)(II) -- Cyclopentanone sodium bisulfite (2.26 g, 12 mmoles) in water (3 ml) was cooled in an ice bath and treated with vigorous stirring with potassium cyanide-^{14}C (643 mg, 9.9 mmoles, 25.8 mCi). The mixture was stirred at 5° for 2 hr. To the mixture was added sodium chloride (4 g) and water (10 ml), and the mixture was extracted with ether. The extract was washed with water saturated with sodium chloride, dried over sodium sulfate and evaporated to give 1-hydroxycyclopentanecarbonitrile-

(nitrile-^{14}C)(1.09 g, 25.5 mCi); νmax (cm^{-1}, liquid film): 3200 (OH), 2200 (CN).

1-Acetoxycyclopentanecarbonitrile-(nitrile-^{14}C)(III) -- A mixture of 1-hydroxycyclopentanecarbonitrile-^{14}C (II)(1.09 g, 9.8 mmoles, 25.5 mCi), acetyl chloride (0.3 ml) and acetic anhydride (30 ml) was heated under reflux for 3 hr. After cooling, the solution was neutralized with 15% sodium bicarbonate aqueous solution and extracted with ether. The ethereal extract was dried over sodium sulfate and evaporated to afford 1-acetoxycyclopentanecarbonitrile-(nitrile-^{14}C) (1.41 g, 24.0 mCi); νmax (cm^{-1}, liquid film): 1735 (CO), 2220 (CN).

1-Aminomethyl-1-hydroxycyclopentane-(aminomethyl-^{14}C)(IV) -- To a solution of 1-acetoxycyclopentanecarbonitrile-^{14}C (III)(1.41 g, 9.2 mmoles, 24.0 mCi) in anhydrous ether (30 ml) was slowly added lithium aluminum hydride (0.76 g, 20 mmoles) at 5-10°. The mixture was stirred at room temperature for 1 hr and subsequently heated to reflux for 3 hr. After degradation of the excess reagent with aqueous ether, 50% potassium hydroxide aqueous solution (30 ml) was added to the mixture. The mixture was continuously extracted with ether by a Kutscher-Steudel extractor for 17 hr. The ethereal extract was dried over potassium carbonate and evaporated to give 1-aminomethyl-1-hydroxycyclopentane-(aminomethyl-^{14}C)(0.79 g, 17.8 mCi); its IR spectrum was identical with that of the unlabelled authentic sample.

Cyclohexanone-2-^{14}C (V) -- To a solution of 1-aminomethyl-1-hydroxycyclopentane-(aminomethyl-^{14}C)(IV)(0.79 g, 6.9 mmoles, 17.8 mCi) in 25% acetic acid (12 ml) was added dropwise a solution of sodium nitrite (1.04 g, 15 mmoles) in water (2.5 ml) at -5°. The mixture was stirred at room temperature for 0.5 hr and then at 60° for 2 hr. After cooling, the mixture was neutralized with 25% sodium carbonate and then saturated with sodium chloride. The mixture was extracted with ether. The aqueous layer was further extracted continuously with ether by a Kutscher-Steudel extractor for 6 hr. The combined ethereal extract was dried over sodium sulfate and concentrated to nearly 2 ml. To the solution was added 40% sodium bisulfite aqueous solution (2 ml) and the mixture

stirred in an ice bath for 8 hr. Filtration of the precipitate produced gave a sodium bisulfite salt of cyclohexanone-2-^{14}C which was recrystallized from 50% ethanol-water. After filtration, the crystalline product was dissolved in 10% sodium hydroxide solution (15 ml) and extracted with ether by a Kutscher-Steudel extractor for 8 hr. The ethereal extract was dried over sodium sulfate and evaporated to give cyclohexanone-2-^{14}C (392 mg, 10.4 mCi); as evidenced by radio gaschromatography its radiochemical and chemical purity was found to be 99%, and its IR spectrum (liquid film) showed an absorption at 1710 cm^{-1} (CO) and was identical with that of the unlabelled authentic sample.

1,1-Bis(4-hydroxyphenyl)cyclohexane-2-^{14}C (VI) -- A mixture of cyclohexanone-2-^{14}C (392 mg, 4.0 mmoles, 10.4 mCi), phenol (3.76 g, 40 mmoles), ethyl mercaptan (230 mg, 3.7 mmoles), concentrated hydrochloric acid (1 drop) and benzene (3 ml) was heated in a sealed ampoule at 70° for 3 hr. After cooling, the crystalline product was collected by filtration and washed with a little amount of chilled benzene to give 1,1-bis(4-hydroxyphenyl)cyclohexane-2-^{14}C (VI)(868 mg, 8.41 mCi); mp. 190-192°, identical in every respect with the unlabelled authentic sample.

1,1-Bis[4-(1-carboxy-1-methylpropoxy)phenyl]cyclohexane-2-^{14}C (S-8527-2-^{14}C)(I) -- To a solution of 1,1-bis(4-hydroxyphenyl)cyclohexane-2-^{14}C (VI)(867 mg, 3.2 mmoles, 8.41 mCi) in ethyl methyl ketone (18 ml) was added portionwise powdered potassium hydroxide (4.81 g, 88 mmoles) at 0-5° and the mixture stirred at 15-20° for 0.5 hr. To the mixture was added a solution of chloroform (2 ml, 24 mmoles) in ethyl methyl ketone (2 ml) with vigorous stirring during 1 hr at 15-20°. The reaction mixture was stirred at the same temperature for 3 hr and heated to reflux for 1 hr. After cooling, the mixture was fractionated by the procedure schematically shown in Fig. 2; giving three fractions (F1, F2 and F3). Evaporation of F1 gave a crystalline residue which was recrystallized from isopropyl alcohol to recover 1,1-bis(4-hydroxyphenyl)cyclohexane-2-^{14}C (VI) in 10% yield (89 mg, 0.86 mCi); identical in every respect with the authentic sample. After concentration of F2, the oily residue obtained was chromatographed over silica gel and eluted with 3% methanol-chloroform. Evaporation of the first

eluate afforded a crystalline residue which was recrystallized from benzene-*n*-hexane to give 1-[4-(1-carboxy-1-methylpropoxy)phenyl]-1-cyclohexene-2(6)-^{14}C (VIII)(28 mg, 0.26 mCi, 3.1% from VI); mp. and mixed mp. 90-92°, identical in all respects with the unlabelled authentic sample. Evaporation of the second eluate followed by recrystallization from chloroform gave 1-[4-(1-carboxy-1-methylpropoxy)phenyl]-1-(2-oxo-3-ethyl-3-methylcoumaran-5-yl)cyclohexane-2-^{14}C (IX)(16 mg, 0.09 mCi, 1.1%); mp. and mixed mp. 160-163°, identical in every respect with the unlabelled authentic sample. The third eluate, after recrystallization from chloroform, afforded 1-[4-(1-carboxy-1-methylpropoxy)phenyl]-1-(4-hydroxyphenyl)cyclohexane-2-^{14}C (VII)(29 mg, 0.20 mCi, 2.4%); mp. and mixed mp. 175-177°, identical in all respects with the unlabelled authentic sample. F3 was evaporated to leave a crystalline residue. Column chromatography of the residue on silica gel with 3% methanol-chloroform followed by recrystallization of the main product from benzene-*n*-hexane gave 1,1-bis[4-(1-carboxy-1-methylpropoxy)phenyl]cyclohexane-2-^{14}C (I)(562 mg, 3.11 mCi, 37%); mp. and mixed mp. 141-144°, the specific activity of 2.59 mCi/mmole, identical in every respect with the unlabelled authentic sample.

Isolation of Unknown A (X) -- The combined mixture of the fraction eluated faster from the column chromatograpy of S-8527-2-^{14}C and the mother liquid obtained from recrystallization was subjected to preparative TLC on precoated silica gel plates (0.25 mm thickness, 20x20 cm, 10 plates, Merck) with chloroform-acetic acid (10:1 v/v) to separate into two radioactive bands. Each band was scraped off from the plates and extracted with 5% acetic acid-chloroform. The extract from the lower R_f-value band afforded 1,1-bis[4-(1-carboxy-1-methylpropoxy)phenyl]cyclohexane-2-^{14}C (235 mg, 1.30 mCi). The other extract from the higher R_f-value band gave unknown A (X)(178 mg, 0.72 mCi, 8.6% from VI) as a pale yellow oil; the radiochemical purity checked by TLC was over 97%.

Esterification of Unknown A (X) -- To a solution of unknown A (96 mg, 0.39 mCi) in ethyl acetate (5 ml) was added an excess of diazomethane in ether and the solution was allowed to stand at room temperature for 15 hr. After addition of

acetic acid to decompose the excess reagent, the solution was washed with 5% sodium carbonate aqueous solution and then water and dried over sodium sulfate. Evaporation of the solvent gave an oily dimethyl ester (XI) in 85% yield (83 mg, 0.33 mCi); νmax (cm^{-1}, liquid film): 1760-1720 (broad and strong) and 1605.

Hydrolysis of Unknown A (X) -- A solution of unknown A (X)(65 mg, 0.26 mCi) in 10% potassium carbonate aqueous solution was heated with stirring at 80-90° for 5 hr. After cooling, the solution was extracted with ethyl acetate and the remaining aqueous solution was acidified with 20% hydrochloric acid. The mixture was extracted with ethyl acetate and the extract washed with water, dried over sodium sulfate and evaporated to give a crystalline residue. Recrystallization of the residue from benzene-*n*-hexane gave 1,1-bis[4-(1-carboxy-1-methylpropoxy)phenyl]cyclohexane-2-^{14}C (I)(42 mg, 0.17 mCi, 65%); mp. and mixed mp. 141-144°, identical in all respects with the authentic sample.

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