LABELLED COMPOUNDS OF POTENTIAL BIOLOGICAL INTEREST

V. Preparation of some hypolipidemic agents labelled with tritium.[+]

Tamas Gosztonyi[++], Göran Bondesson, Karl Erik Domeij and Nils E. Stjernström. Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

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SUMMARY

Two hypolipidemic aryloxyacetic acid derivatives and one aryloxymethylmalonic acid derivative were labelled with tritium in the aryloxy moiety. The corresponding phenols were first labelled by the Yavorsky method and then transformed to the desired products by synthetic reaction steps. For comparative pharmacokinetic and metabolic studies the carboxylic acid corresponding to clofibrate was also labelled by the same procedure.

Key words: Tritium, Exchange labelling, Yavorsky method, Phenols, Aryloxyacetic acid derivatives, Aryloxymethylmalonic acid derivatives, Hypolipidemic agents.

The lipid lowering properties of aryloxyacetic acid derivatives have been extensively studied. One of these compounds, ethyl 2-(4-chlorophenoxy)-2-methylpropionate (clofibrate, Ib) is commonly used in the treatment of hyperlipidemia. It is effective in cases of elevated blood levels of triglycerides, but has a less pronounced effect on high blood levels of cholesterol only.

In the search for compounds more potent than clofibrate a number of dibenzofuranyloxyacetic acid derivatives were prepared (1). One of these, ethyl 2-(4-dibenzofuranyloxy)-2-methylpropionate (IIb), was found to be a very potent hypolipidemic agent in mice and spontaneously hyperlipidemic rats. Like clofibrate, this ester is hydrolysed in vivo to the corresponding acid (IIa) which is responsible for the hypolipidemic effect.

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[++]To whom correspondence should be addressed

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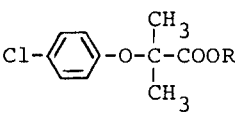
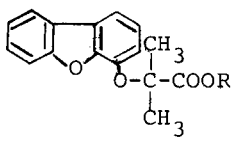
Comparable strong hypolipidemic effects could be observed in the case of ethyl 2-(4-dibenzothiophenoxy)-2-methylpropionate (IIIb) and its corresponding carboxylic acid (IIIa) (2).

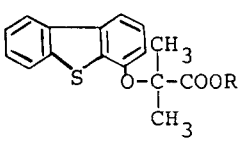
Another class of compounds which was tested for hypolipidemic activity consisted of phenoxymethylmalonic acid esters (3). One of these compounds, ethyl 2-(4-chloro-2-methylphenoxy)-2-methylmalonate (IVb), showed plasma lipid lowering properties of the same magnitude as clofibrate.



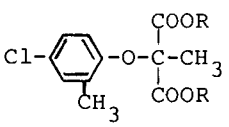
I a R = H
b R = C_2H_5 (clofibrate)



II a R = H
b R = C_2H_5



III a R = H
b R = C_2H_5



IV a R = H
b R = C_2H_5

In order to facilitate pharmacokinetic and metabolic studies with the new compounds (II-IV), they were prepared labelled with tritium in the aromatic moiety. For comparative studies 2-(4-chlorophenoxy)-2-methylpropionic acid (Ia) labelled with tritium was also synthesized.

In the first step in the preparation of the tritium-labelled compounds the corresponding phenols were tritiated. For this purpose the method of Yavorsky and Gorin (4) proved to be useful. This method is currently used in our laboratory for the preparation of compounds generally labelled with tritium (5). The results of tritium labelling of the phenols are summarized in Table 1.

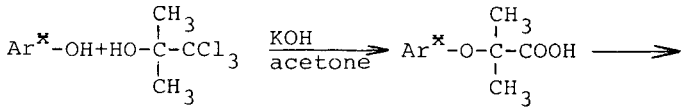
Table 1. Results of tritium labelling of the phenolic components by the Yavorsky method.

Phenol			$H_2TPO_4 \cdot BF_3$		$\frac{W_S}{W_R}$	Reaction		Product+		
No	Name	weight w_s mg	weight w_R mg	Spec.act. μCi/mg		Time hrs.	Temp. °C	Yield %	Spec.act. μCi/mg	Spec.act. mCi/mM
1	4-chloro-phenol	700	3 000	800	0.234	64	80	12	374	48
2	4-hydroxy-dibenzo-furan	500	3 000	782	0.166	6	25	100	400	73.6
3	4-hydroxy-dibenzo-thiophene	500	2 100	757	0.238	6	25	86	41	8.2
4	4-chloro-2-methyl-phenol	600	2 700	757	0.222	48	80	38	522	75

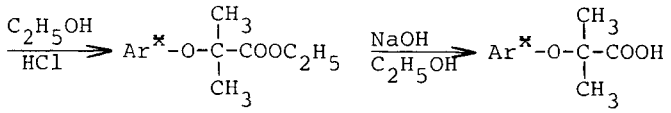
+Yields and specific activity values are given for the crude products which were reacted further without purification.

The labelled phenols 1-3 were reacted with 1,1,1-trichloro-2-methyl-2-propanol to give the acids Ia-IIIa. The acids IIa and IIIa were difficult to purify directly and obtain in crystalline form, especially on the small scale used in the isotopic syntheses. Therefore they were transformed to their ethyl esters (IIb and IIIb) which after purification were hydrolyzed to the acids again. However, the acid Ia could be obtained in a pure state without being first transformed to the ester. The principles mentioned above are illustrated by equation 1.

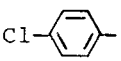
For the preparation of IVa and IVb the labelled phenol 4 was reacted with diethyl 2-chloro-2-methylmalonate in dimethyl sulfoxide in the presence of sodium hydroxide. The ester IVb was formed as a yellow oil after purification on a silica gel column and was hydrolyzed to the corresponding acid according to equation 2.

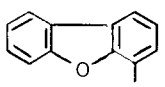


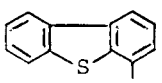
1-3 Ia, IIa, IIIa



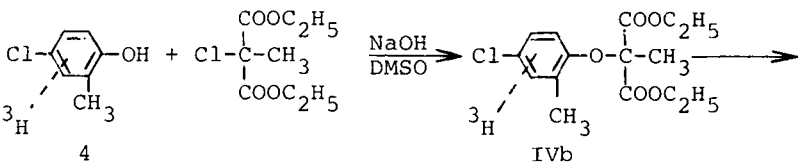
IIb, IIIb IIa, IIIa

I Ar^{*}=  ^{3}H labelled

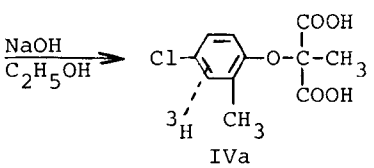
II Ar^{*}=  ^{3}H labelled

III Ar^{*}=  ^{3}H labelled

(equation 1)



4 IVb



IVa

(equation 2)

The radiochemical purity of the labelled compounds was checked by thin layer chromatography and was found to be better than 95 % in all cases. Due to the relatively low specific activities the products could be stored for several months at room temperature without decomposition.

EXPERIMENTAL

Melting points reported are uncorrected.
Specific activity of the labelled compounds was measured in a Packard TriCarb liquid scintillation spectrometer (Model 3320) using internal standardization (Hexadecane-1,2-^{3}H from the Radiochemical Centre, Amersham).

Thin layer chromatography. Precoated silica gel plates (F_{254}, 0.25 mm layer thickness, E.Merck, Darmstadt) were used. Spots were detected by visual examination under UV-light. The distribution of radioactivity along the chromatogram was determined by the use of a Berthold "Dünnschicht Scanner II".

Labelling of the phenols. The boron trifluoride complex of tritiated phosphoric acid (Yavorsky reagent) was prepared as described previously (5). The experimental conditions and results of the labelling reactions are summarized in Table 1.

2-[4-Chlorophenoxy-^{3}H(G)]-2-methylpropionic acid (Ia). - Crude tritiated p-chlorphenol (84 mg, 0.65 mM) was dissolved in 5 ml of acetone and solid potassium hydroxide (224 mg, 4 mM) was added. A solution of 1,1,1-trichloro-2-methyl-2-propanol (187 mg, 1 mM) in 5 ml of acetone was then added and the mixture was heated under reflux for two hours. A thick white precipitate was formed. The solvent was removed under reduced pressure and the residue was dissolved in 2 ml of water. The aqueous solution was washed with ether to remove any unreacted phenol and then acidified with a few drops of concentrated hydrochloric acid, whereupon the acid Ia precipitated. The mixture was extracted with ether, the solvent was removed at reduced pressure and the residue was washed with 1 ml of petroleum ether (bp. 40-60^{o}C). The crude product having a specific activity of 143 μCi/mg (26 mCi/mM) was diluted with 50 mg of pure inactive carrier and was recrystallized first from benzene-petroleum ether and then from ethanol-water. Yield: 60 mg white crystalline product. Mp.: 118-120^{o}C. Specific activity: 24 μCi/mg = 4.4 mCi/mM. TLC in n-heptane-chloroform-acetone-acetic acid (14:2:2:2) showed a single radioactive peak at R_F = 0.35 corresponding to the R_F value of an authentic sample.

Ethyl 2-[4-dibenzofuranyloxy-^{3}H(G)]-2-methylpropionate (IIb). - Crude tritiated 4-hydroxydibenzofuran (500 mg, 2.7 mM) was dissolved in 10 ml of acetone and solid potassium hydroxide (900 mg, 16 mM) was added. A solution of 1,1,1-trichloro-2-methyl-2-propanol (750 mg, 4 mM) in 5 ml of acetone was then added and the mixture was heated under reflux for two hours. The solvent was removed under reduced pressure and the residue was dissolved in 5 ml of water. After washing the aqueous solution with ether it was acidified with a few drops of concentrated hydrochloric acid. Extraction with ether and removal of the solvent under reduced pressure gave the crude acid IIa which was dissolved in 5 ml of 95 % ethanol. Ethanol saturated with hydrogen chloride (20 ml) was then added and the mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure and the residue was dissolved in ether. The ethereal solution was washed with saturated aqueous $NaHCO_3$ and then evaporated to dryness. The residue was crystallized from n-hexane yielding 236 mg of the ester IIb (0.8 mM = 30 % based on the crude phenol). Mp.: 59-61°C. Specific activity: 293 μCi/mg = 87 mCi/mM. TLC in benzene-ethanol (18:2) showed a single radioactive peak at R_F= 0.62 which corresponds to the R_F value of the authentic ester.

2-[4-Dibenzofuranyloxy-^{3}H(G)]-2-methylpropionic acid (IIa). - The ester IIb (44 mg) was diluted with 400 mg of inactive ester and dissolved in 4 ml of absolute ethanol. After adding 0.5 ml of 30 % aqueous NaOH the mixture was heated on a water bath at 80°C for two hours. After cooling the ethanol was removed under reduced pressure and the residue was dissolved in 3 ml of water. The solution was acidified with concentrated hydrochloric acid and the product was extracted with ether. Evaporation to dryness gave a yellow oil which soon solidified. Recrystallization from ethanol-water gave 326 mg white crystals (80 %). Mp.: 126.5-128°C. Specific activity: 30 μCi/mg = 8.1 mCi/mM. TLC in n-heptane-chloroform-acetone-acetic acid (14:2:2:2) showed a single radioactive peak at R_F= 0.26 which corresponds to the R_F value of an authentic sample.

2-[4-Dibenzothiophenoxy-^{3}H(G)]-2-methylpropionic acid (IIIa) was prepared via the ester essentially by the same methods as described above. The pure acid was obtained in a yield of 21 % based on crude 4-hydroxydibenzothiophene-^{3}H(G). Mp.: 114-116°C. Specific activity:

17.6 μCi/mg = 5 mCi/mM. TLC in n-heptane-chloroform-acetone-acetic acid (14:2:2:2) showed a single radioactive peak at R_F= 0.27 which corresponds to the R_F value of an authentic sample.

Diethyl 2-[4-chloro-2-methylphenoxy-^{3}H(G)]-2-methylmalonate (IVb). - Crude tritiated 4-chloro-2-methyl phenol (288 mg, 1.6 mM) was dissolved in a mixture of 1 ml of dimethyl sulfoxide and 4 ml of benzene. Pulverized sodium hydroxide (66 mg, 1.6 mM) was added and the benzene was evaporated under reduced pressure in order to remove the water formed during the formation of the phenolate. Benzene (4 ml) was then added again and diethyl 2-chloro-2-methylmalonate (334 mg, 1.6 mM) was added dropwise at room temperature. The mixture was then heated at 80°C for 20 hours. After cooling it was poured into 20 ml of ice water and extracted with four 5 ml portions of ether. TLC in chloroform-acetone (19:1) showed that the ethereal extract contained two radioactive components. About 70 % of the radioactivity could be localized in a spot at R_F= 0.65 corresponding to the R_F value of an authentic sample of the desired product, while 30 % of the radioactivity was bound to the unreacted phenol (R_F= 0.45). The mixture was separated on a silica gel column (40 g of silica gel in toluene, 15 mm diam.) by elution with a gradient of toluene and a mixture of chloroform-acetone (19:1). The ester was obtained as a colourless oil. Yield: 105 mg (20 %). Specific activity: 206 μCi/mg = 64.8 mCi/mM.

2-[4-Chloro-2-methylphenoxy-^{3}H(G)]-2-methylmalonic acid (IVa).- The tritiated ester IVb (5 mg) together with 495 mg of inactive ester was hydrolyzed in 40 ml of ethanol and 2 ml of 30 % aqueous NaOH at room temperature for 24 hours. The ethanol was then removed under reduced pressure, the residue was dissolved in water and washed with ether. The aqueous solution was acidified with concentrated hydrochloric acid and extracted with ether. After drying ($MgSO_4$) the ether was removed under reduced pressure yielding 400 mg of a colourless oil (97 %). Specific activity: 2.6 uCi/mg = 673 μCi/mM. TLC in n-heptane-chloroform-acetone-acetic acid (14:2:2:2) showed a single radioactive peak at R_F= 0.1 which corresponds to the R_F value of an authentic sample of the acid.

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