SYNTHESIS OF ^{14}C-BUCROMARONE SUCCINATE AND HYDROCHLORIDE

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SUMMARY

^{14}C-Bucromarone, 2-[4-(3-N,N dibutylamino propoxy) 3,5-dimethyl benzoyl] chromone, was synthesized from (U-^{14}C) oxalic acid. The labelling takes place at the first step of the synthesis, and allowed the obtention of

-^{14}C-Bucromarone succinate, with a specific activity of 7.45 mCi/mmol, i.e. 275.6 MBq/mmol ;

-^{14}C-Bucromarone hydrochloride, with a specific activity of 7.5 mCi/mmol, i.e. 277.5 MBq/mmol.

Key words : ^{14}C-Bucromarone succinate and hydrochloride
^{14}C-2-Aroyl chromone derivatives

Bucromarone, 2-[4-(3-N,N dibutylamino propoxy) 3,5-dimethyl benzoyl] chromone 8 (1), exhibits some chemical features similar to those of Amiodarone, an anti-angorous and anti-arrythmic drug of therapeutical use. The anti-arrythmic properties of Bucromarone were studied in a work devoted to the pharmacological properties of aroyl-chromones (2). Thus it was of interest to examinate the "in vivo" biodisposition and metabolism of this molecule.

With this object, we undertook the ^{14}C-labelling of Bucromarone at both the 2-position of the chromone ring and the carbonyl function of the benzoyl group, using (U-^{14}C) oxalic acid as the radioactive precursor (cf. Scheme).

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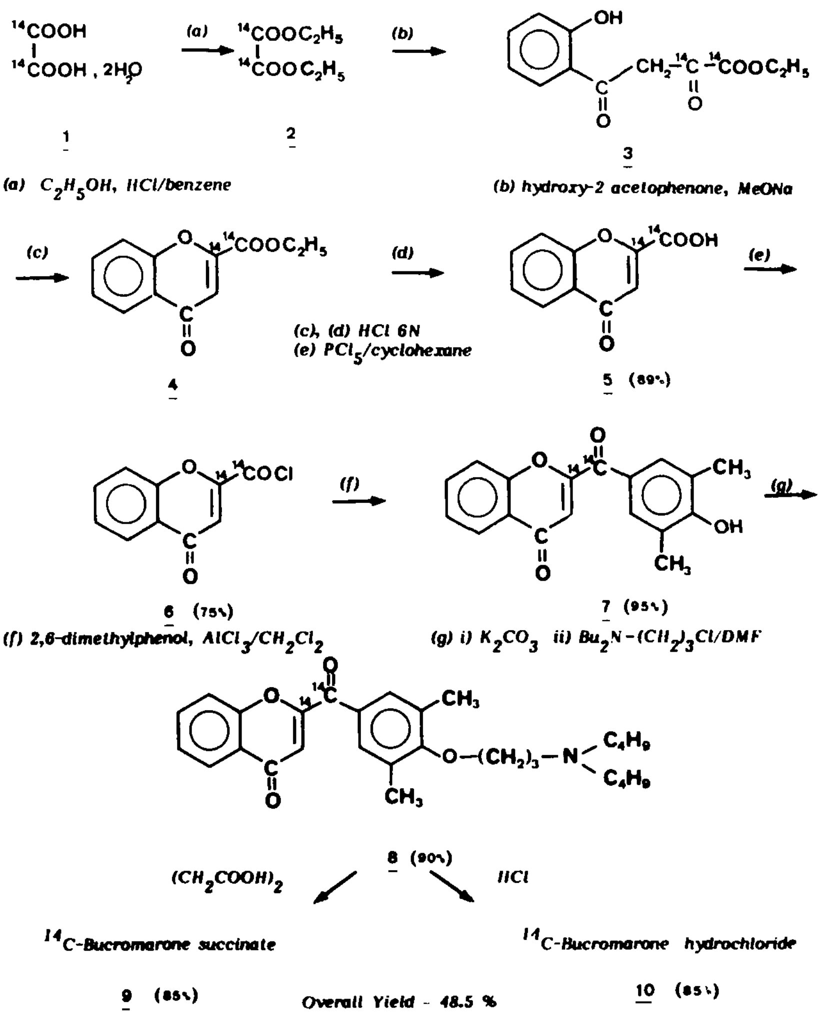
SCHEME



The chemical yields in Bucromarone succinate 9 and Bucromarone hydrochloride 10 were both 48.5 %, Bucromarone itself being obtained in a 57 % yield.

Thus, starting from 3 mmol (35 mCi) of (U-^{14}C) oxalic acid, the actual synthesis allowed the obtention of :

- 0.68 mmole of ^{14}C-Bucromarone succinate (specific activity : 7.45 mCi/mmol)

- 0.66 mmole of ^{14}C-Bucromarone hydrochloride (specific activity 7.5 mCi/mmol).

EXPERIMENTAL

Thin layer radiochromatograms were performed through silica gel (MERCK 60 F 254/ref. 5719) and analyzed with a multi-channel detector BERTHOLD LB 2821.

NMR spectra were recorded on a JEOL C 60 H apparatus (60 MHz). Chemical shifts are given in ppm (TMS = 0).

Table of Rf values

Compound	**Solvent**	**Rf**	**Revelator**
2	$CHCl_3$	0.50	UV
5	$CHCl_3$,CH_3OH,HCOOH/90,7,3	0.20	UV Iodine
	$CHCl_3$,C_2H_5OH/95,5	0.30	UV Iodine
7	$CHCl_3$,CH_3OH,HCOOH/90,7,3	0.70	UV Iodine
	Toluene, Dioxan, CH_3COOH/10,6,1	0.68	UV Iodine
8	$CHCl_3$,CH_3OH,HCOOH/90,7,3	0.38	UV Iodine
	Toluene, Dioxan, CH_3COOH/10,6,1	0.52	UV Iodine
9	Toluene,Dioxan,CH_3COOH/10,6,1	0.40	UV Iodine
10	Toluene,Dioxan,CH_3COOH/10,6,1	0.40	UV Iodine

I. Ethyl ^{14}C-oxalate 2

The labelled precursor (U-14C) oxalic acid was available from AMERSHAM, as $(COOH)_2$,$2H_2O$, with a specific radioactivity of 108 mCi/mmol. The required activity for ^{14}C-Bucromarone 8 being 20 µCi/mg (9.25 mCi/mmol), the radiochemical synthesis was carried out from 35 mCi (U-^{14}C)-$(COOH)_2$, diluted with a suitable amount of unlabelled reagent.

(U-^{14}C) oxalic acid, $2H_2O$ (35 mCi) was delivered as a lyophilized powder in sealed ampoules, the contents of which were dissolved in acetone, the solution was transferred, then added with 357 mg of unlabelled oxalic acid, $2H_2O$, and finally evaporated.

The residue (378 mg = 3 mmol. ^{14}C-$(COOH)_2$, $2H_2O$ 1) is diluted with 5 ml absolute EtOH, 0.5 ml 37 % HCl, 50 ml benzene, and the mixture *is heated in a DEAN-STARK separator, until its volume is reduced to ca. 20 ml.*

A radiochromatographic control of the mixture allows the calculation of the percentage of ethyl oxalate 2. To be brought to completion, the esterification may need a further addition of some HCl and EtOH.

Crude ethyl ^{14}C-oxalate 2 is obtained from evaporation of the solvents with trivial loss, owing to its relatively high boiling point (b.p. = 185-6°C/760 torrs). The radiochemical purity is 97 % (1 being the main impurity).

NMR ($CDCl_3$) CH_3-CH_2-OO^{14}C-^{14}COO-CH_2-CH_3

δ(ppm) : 1.40(t,J = 7 Hz,6H = 2CH_3) ; 4.15 (q, J = 7 Hz, 4H = 2CH_2)

M.W. = 146

II. Ethyl o.hydroxy benzoyl ^{14}C-pyruvate (sodium salt) 3.

In a 10 ml long-necked flask, connected to a lateral collector, sodium methoxide is prepared from sodium (230 mg, 10 mmol) and MeOH (2.5 ml). After complete disappearing of the metal, excess MeOH is evaporated by heating first under normal pressure, then under vacuum ; hexane (3 ml) is added, and the mixture is heated on stirring, so as to scatter the precipitate, while hexane is distilled under normal pressure, then under vacuum. Finally 3 ml hexane are added, and the methoxide stirred and scattered as above.

In the flask containing ^{14}C-ethyl oxalate, o-hydroxy acetophenone (448 mg ; 3.3 mmol.) and hexane (1 ml) are placed, and this solution is added dropwise to the methoxide, while the mixture is cooled at room temperature with a water bath. The slurry turns from yellow to orange colour. 5 minutes past the end of the addition, hexane is distilled under normal pressure, by heating with an oil bath.

The reaction is made complete by a further addition of 58 mg (0.4 mmol) of unlabelled ethyl oxalate, dissolved in 1 ml hexane. The solvent is distilled as above, and the residue evaporated to dryness under vacuum.

The sodium salt of ethyl o-hydroxy-benzoyl^{14}C-pyruvate 3 is obtained as an orange-yellow powder, and used as such, without any further purification.

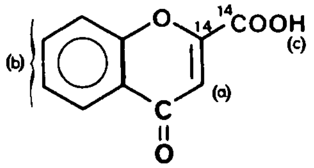
III. Ethyl ^{14}C-2-chromone carboxylate 4 and ^{14}C-2-chromone carboxylic acid 5.

To the crude salt 3, 1 ml EtOH and 1 ml 37 % HCl are first added, and the mixture is stirred and heated (oil bath) until most of EtOH is distilled. Hydrolysis is achieved by addition of 37 % HCl (1.5 ml) and H_2O (2 ml), and heating the mixture 2 hrs at 100°C.

After cooling and addition of crushed ice, the white precipitate is filtered and washed with icy water, then dried under vacuum and over P_4O_{10}.

2-chromone carboxylic acid 5 (m.p. = 254-6°C) is obtained in *a 89 % yield, with a radiochemical purity of 96 %.*

NMR (D_6DMSO) :



M.W. = 190

δ(ppm) : 6.90 (s, 1H = a) ; 7.35-8.15 (m,4H = b) ; 12 (s, 1H = c)

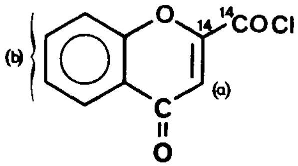
*IV. ^{14}C-2-chromone carboxylic acide chloride **6** (3)*

Into a 20 ml flask, fitted with a refluxing condenser, compound 5 (550 mg ; 2.89 mmol.) is introduced with PCl_5 (662 mg : 3.18 mmol.) and cyclohexane (7 ml) (freshly distilled over sodium metal). The mixture is refluxed to complete dissolving (ca 15 minutes) and 10 minutes more, then allowed to cooling.

The yellowish precipitate is filtered and washed with a very little (ca 1 ml) of cold cyclohexane.

Yield : 75 %   m.p. = 106-7°C

NMR ($CDCl_3$) :



M.W. = 208.5

δ(ppm) : 6.70 (s, 1H = a) ; (7.20-8.20 (m, 4H = b)

V. ^{14}C 2-(3,5 dimethyl 4-hydroxy benzoyl) chromone 7

A mixture of 2,6 dimethyl phenol (450 mg ; 2,15 mmol.), $AlCl_3$ (860 mg ; 6.45 mmol.) and CH_2Cl_2 (5 ml) is stirred 15 minutes at room temperature, in a 20 ml flask protected by a $CaCl_2$ trap.

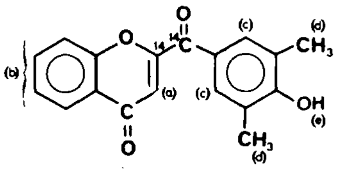
After cooling at 0°C, a solution of acid chloride 6 (450 mg ; 2.15 mmol.) in 5 ml CH_2Cl_2 is added dropwise. Stirring is kept on for 3 hrs at 0°C, then overnight at room temperature.

Most of the solvent is evaporated, and 10 ml of icy HCl (10 %) are added on cooling with an ice bath. The yellow-ochre precipitate is filtered, washed with water, dried and crystallized from 20 ml of a mixture acetone/ ethanol 1/4. Evaporating the solution to half-volume yields compound 7 as light yellow needles, m.p. = 215°C (Th. 217).

Yield : 600 mg (95 %)

The radiochemical purity of 7 is 95.5 % (radiochromatography)

NMR (D_6DMSO) :



M.W. = 294

δ (ppm) : 2.25 (s,6H = d); 6.70 (s, 1H = a) ; 7.30-8.30 (m, 6H = b + c) ; 9.55 (s,1H = e)

VI. ^{14}C-2-[4-(3-N,N dibutylamino propoxy) 3,5-dimethyl benzoyl] chromone or Bucromarone 8

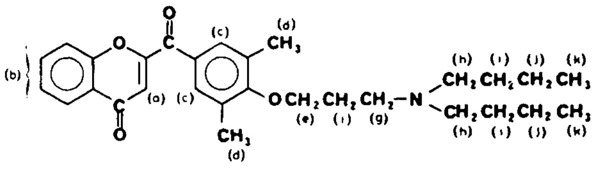
A mixture of 7 (530 mg ; 1.8 mmol.), potassium carbonate (125 mg ; 0.9 mmol.) and DMF (5 ml) is heated 1/2 hr at 100°C. Then 3-(N,N dibutylamino) 1-chloro propane (370 mg ; 1.8 mmol.) dissolved in 2 ml DMF, is added, and the mixture is heated 2 hrs at 100°C.

When the reaction is brought to completion (as indicated by radiochromatography), the solution is cooled, filtered and evaporated under vacuum. The residue is taken on with water and benzene, and the aqueous layer extracted twice with benzene. The extracts are dried over $MgSO_4$ and evaporated.

A radiochromatographic control of the crude product indicates that 8 is soiled with little amounts of unreacted 7, and with some non-radioactive impurities. The sample is purified by chromatography through silica-gel 60 (230-400 Mesh, MERCK ref. 9385). The main impurities are first eluted by pure CH_2Cl_2, and then bucromarone 8 is eluted by a mixture CH_2Cl_2/ EtOH (95/5).

Bucromarone 8 is obtained as an orange-yellow syrup, with a radiochemical purity of 96 % (yield : 90 %).

NMR ($CDCl_3$) :



M.W. = 463.6

δ (ppm) : 0.80-1.10 (t, J = 6Hz, 6H = k) ; 1.10-1.65 (m, 8H = i + j); 1.70-2.25

(m, 2H = f) ; 2.35 (s, 6H = d) ; 2.43-2.86 (m, 6H = g + h) ; 3.80-4.05 (t, J = 7 Hz 2H = e) ; 6.80 (s, 1 H = a) ; 7.25-8.40 (m, 6H = b + c).

*VII. ^{14}C-Bucromarone succinate **9***

In a 10 ml flask, succinic acid (131 mg ; 1.11 mmol.) is mixed with ethyl acetate (2 ml), in which the most part of the acid remains insoluble.

A solution of bucromarone 8 (367 mg ; 0.792 mmol.) is added, and the mixture is heated with an oil bath at 80°C, on hand-stirring, the flask being protected by a $CaCl_2$ trap.

Dissolution of succinic acid occurs progressively, and is complete over ca 10 minutes. Then the homogeneous solution is cooled at -5°C, and crystallization is initiated by vigorous scratching. The crystals are filtered, washed twice with diethyl ether, and dried under vacuum over P_4O_{10}.

^{14}C-Bucromarone succinate 9 is obtained as a white powder (395 mg 85 %) m.p. = 95°C.

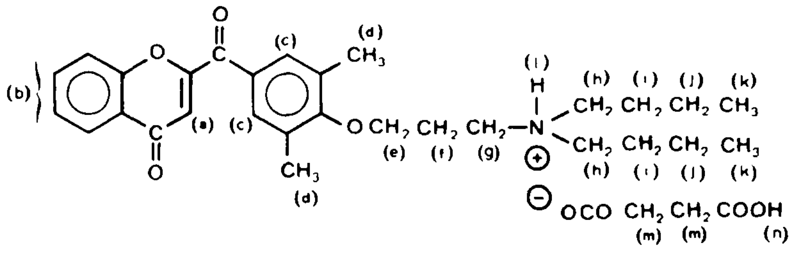
Radiochemical purity : 98.2 % (impurities are 7 1 % and 4-(3-N butylamino propyl) 3,5-dimethyl 2-benzoyl chromone 0.8 %).

Specific radioactivity : 12.8 µCi/mg (7.45 mCi/mmol.)

The isolated salt contains about 1.5 g per 100 g of free succinic acid.

The latter is titrated by HPLC on a 10 µ Bondapak C 18 Column (Waters), UV detector : 214 nm. The eluent is a 0.05 M phosphate buffer (pH 2.5), with a 2 ml/min. flow rate.

NMR (D_6 Acetone) :



M.W. = 581.7

δ (ppm) : 0.80-1.10 (t,J = 6 Hz, 6H = k) ; 1.15-2.60 (m, 10H = i + j + f); 2.30 (s, 6H = d) ; 2.45 (s, 4H = m) ; 2.85-3.60 (m, 6H = g + h) ; 3.9-4.2 (t, J 6 Hz, 2H = e) ; 6.65 (s, 1H = a) ; 7.25-8.20 (m, 6H = b + c) ; 9.6 (s, 2H = l + n).

*VIII. ^{14}C-Bucromarone hydrochloride **10***

To a solution of ^{14}C-Bucromarone (359 mg, 0.775 mmol.) in dry benzene (5 ml), a 2 N solution of HCl gas in anhydrous ether is added.

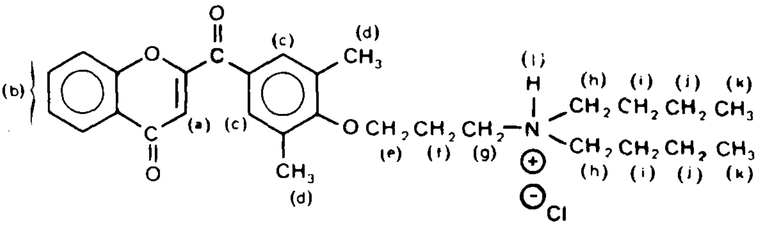
The hydrochloride 10 appears first as a syrupy precipitate. Traces of moisture are best eliminated by successive addition and evaporation of anhydrous ethyl acetate. Finally the residue is taken on with 1 ml abs. EtOH, and precipitated with 50 ml dry Et$_2$O. The flocculent white precipitate is filtered, washed with ether and dried.

Yield : 330 mg (85 %)

Radiochemical purity 99.6 % (impurity : 7 : 0.4 %)

Specific radioactivity 15 µCi/mg (7.5 mCi/mmol.)

NMR (D$_6$ Acetone) :



M.W. = 500.0

δ (ppm) : 0.80-1.10 (t, J = 6Hz, 6H = k) ; 1.20-2.70 (m, 10H = i + j + f) ; 2.35 (s, 6H = d) ; 2.85-3.60 (m, 6H = g + h) ; 3.9-4.2 (t, J = 6Hz, 2H = e) ; 6.65 (s, 1H = a) ; 7.30-8.15 (m, 6H = b + c) , 12.7 (s, 1H = l).

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