# SYNTHESIS OF DEUTERIUM AND TRITIUM LABELLED
## DERIVATIVES OF MALEOPIMARIC ACID

Peter J. Ramm and John B. Taylor

Roussel Laboratories Limited, Kingfisher Drive, Covingham, Swindon, Wiltshire, SN3 5BZ.   U.K.

## SUMMARY

A new hepatoprotective agent, N-(2-hydroxyethyl)-maleopimarimidyl morpholide (RU18492) has been synthesised labelled with tritium at C-3, C-4a, C-10 and in the C-isopropyl group.   The incorporation of tritium relies on the acid-catalysed isomerisation of levopimaric acid (4) to abietic acid (2), which was then converted in three stages to (3).

## INTRODUCTION

Our investigations into the pharmacological actions of compounds derived from terpenoids, have shown that certain derivatives of maleopimaric acid (1), which is obtained from the diterpene abietic acid (2), have pronounced hepatoprotective activity.   In particular, N-(2-hydroxyethyl)-maleopimarimidyl morpholide (RU18492) at doses of 100-500 mg/kg is capable of reducing serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels to normal in rats challenged with galactosamine, using the method of Keppler[1].   The detailed investigation of RU18492 necessitated the synthesis of radioactively labelled material for metabolic and pharmokinetic studies.

## DISCUSSION

The synthesis of the maleopimaric acid series from abietic acid, a natural product, precluded the possibility of introducing carbon-14-into the nucleus of the molecule.    Labelling with carbon-14 in the pyrrolidinedione or morpholine rings of RU18492 (3), although feasible, was not considered because of their potential lability during metabolism.

The facile isomerisation of levopimaric acid (4) to abietic acid (2) under acid catalysis[2], suggested a convenient means of introducing isotopic hydrogen into the nucleus of the molecule.    When levopimaric acid (4) in dioxan solution was treated with DCl in $D_2O$ at room temperature, the mass spectrum of the deuterated abietic acid (2a) thus obtained, showed it to contain largely $d_1$ material ($d_1$ – 69%; $d_2$ – 27%; $d_3$ – 4%)*.    This was converted to maleopimaric acid (1a) which exhibited an isotopic composition of $d_0$ – 22%; $d_1$ – 51%; $d_2$ – 24%; $d_3$ – 3%, representing a loss of some 20% of deuterium.    These results are consistent with the predominant labelling illustrated in scheme 1, a deuterium atom being incorporated at C-12 during the isomerisation and followed by loss of either deuterium or hydrogen from



**Scheme   1**

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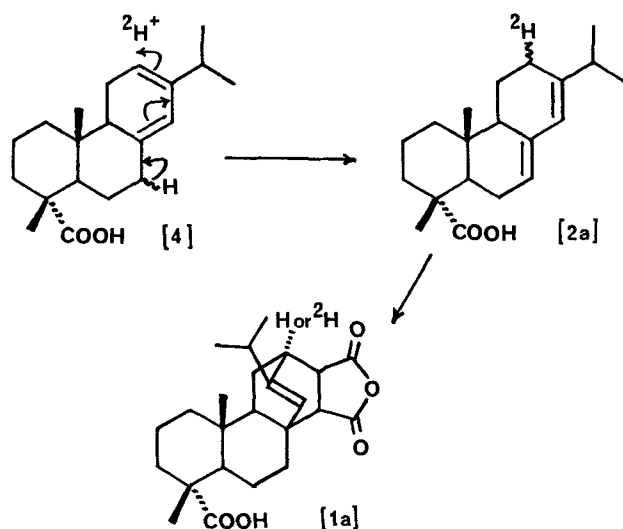
*    The amount of undeuterated acid is not included in the calculations and in each case the same percentage has been deducted from the $d_0$ figure for maleopimaric acid.    In calculating the abundances, allowance was made for the m+1 contribution from the next lower peak.

this position during the formation of maleopimaric acid.    The latter step
must proceed with some degree of stereospecificity since only 20% of
deuterium is lost in the process.

    When the acid-catalysed isomerisation was carried out in refluxing
dioxan, five deuterium atoms were incorporated into the abietic acid (2b)
($d_1$ – 3%; $d_2$ – 3%; $d_3$ – 7%; $d_4$ – 29%; $d_5$ – 58%). *    Comparison of the
n.m.r. spectra of unlabelled abietic acid (2) and labelled material (2b)
(Fig. 1) immediately reveals the locations of two of the deuterium atoms.

Fig. 1
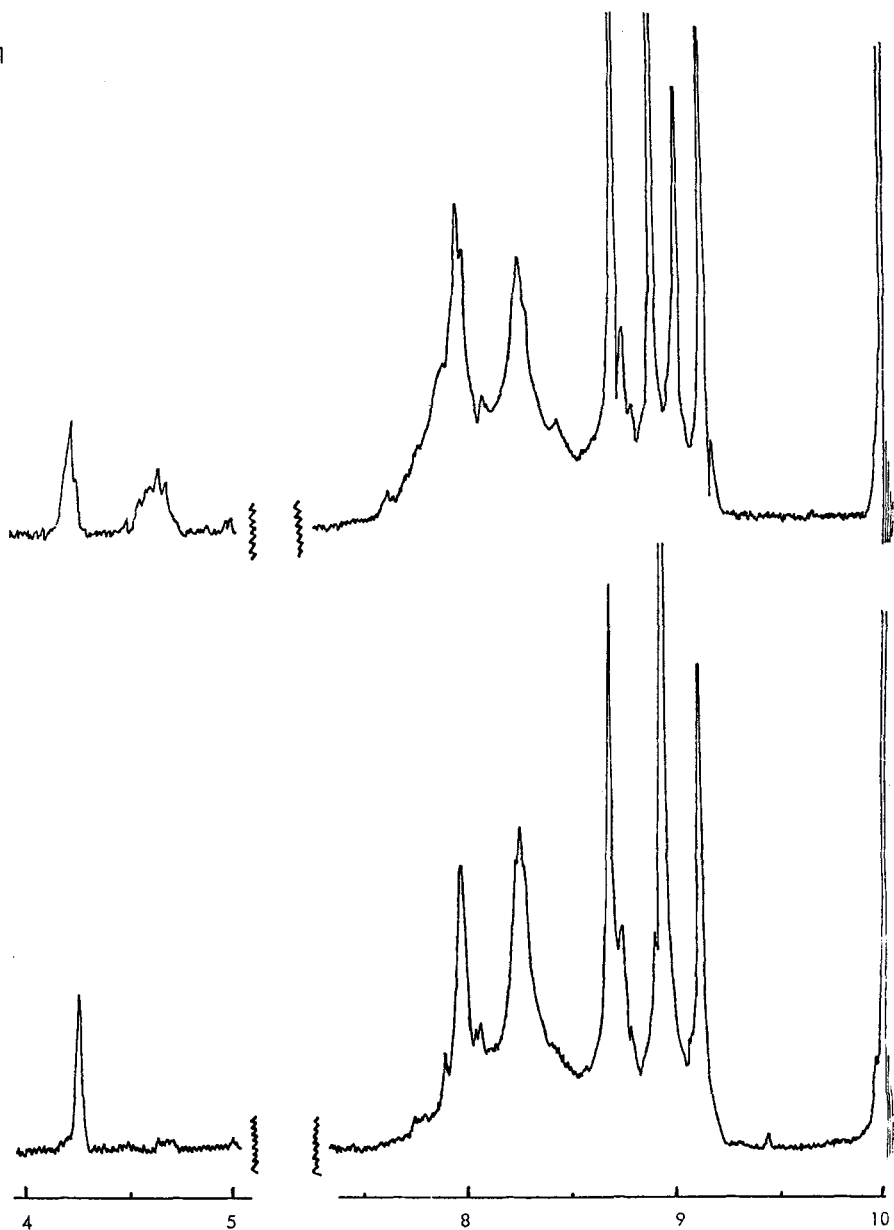
Absence of the signal at 4.6 $\Upsilon$ due to the hydrogen at C-7 is readily

apparent, and in addition, the doublet at 9.1 $\Upsilon$ due to the isopropyl methyl

groups has become a singlet, indicating replacement of the hydrogen at C-15.

The previous experiment had shown the presence of one deuterium at C-12,

and the location of a further deuterium atom was inferred from a publication[3]

which cited both palustric (5) and neoabietic (6) acids as intermediates in the

isomerisation. (Scheme 2)    The presence of palustric acid (5) would lead to

incorporation of deuterium at C-9, whilst that of neoabietic acid (6) would

result in the labelling at C-15 already noted.    The n.m.r. spectrum of (2b)

clearly indicated that C-14 remained unlabelled, suggesting that the

isomerisations occur via fully concerted mechanisms.
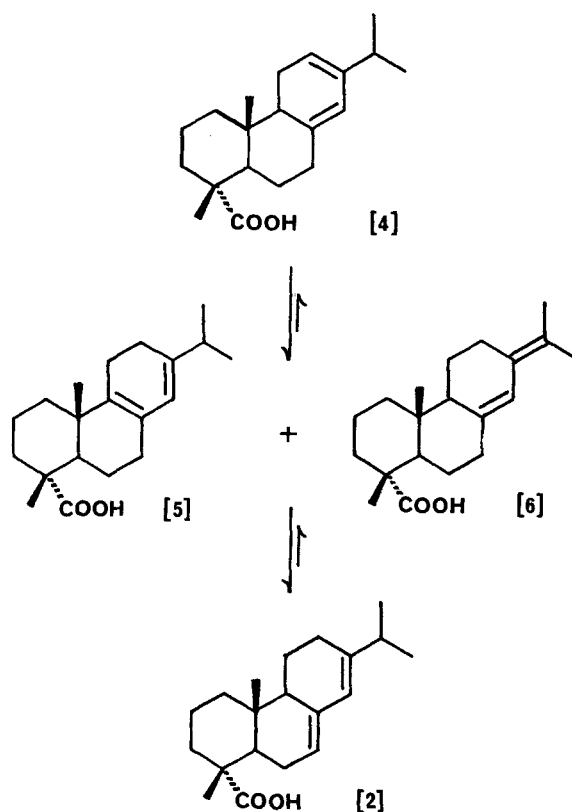


**Scheme 2**

The fifth deuterium atom could most reasonably be accounted for as

a second label at C-12, and further evidence for this assignment was available

when abietic acid (2b) was converted to maleopimaric acid (1b).    Assuming

(2b) contains two deuterium atoms at C-12, one of these must be lost during the formation of maleopimaric acid (1b), irrespective of the stereospecificity of the reaction. The isotopic composition of (1b) was found to be:- $d_o$ - 1%; $d_1$ - 3%; $d_2$ - 12%; $d_3$ - 36%; $d_4$ - 34%; $d_5$ - 14%, indicating a 22% loss of deuterium during the reaction. Although it is clear from these results that not all molecules are losing one deuterium atom, it was felt that the result was of the right order of magnitude to support the argument. Finally, when the n.m.r. spectrum of maleopimaric acid (1) is compared with that of

Fig. 2
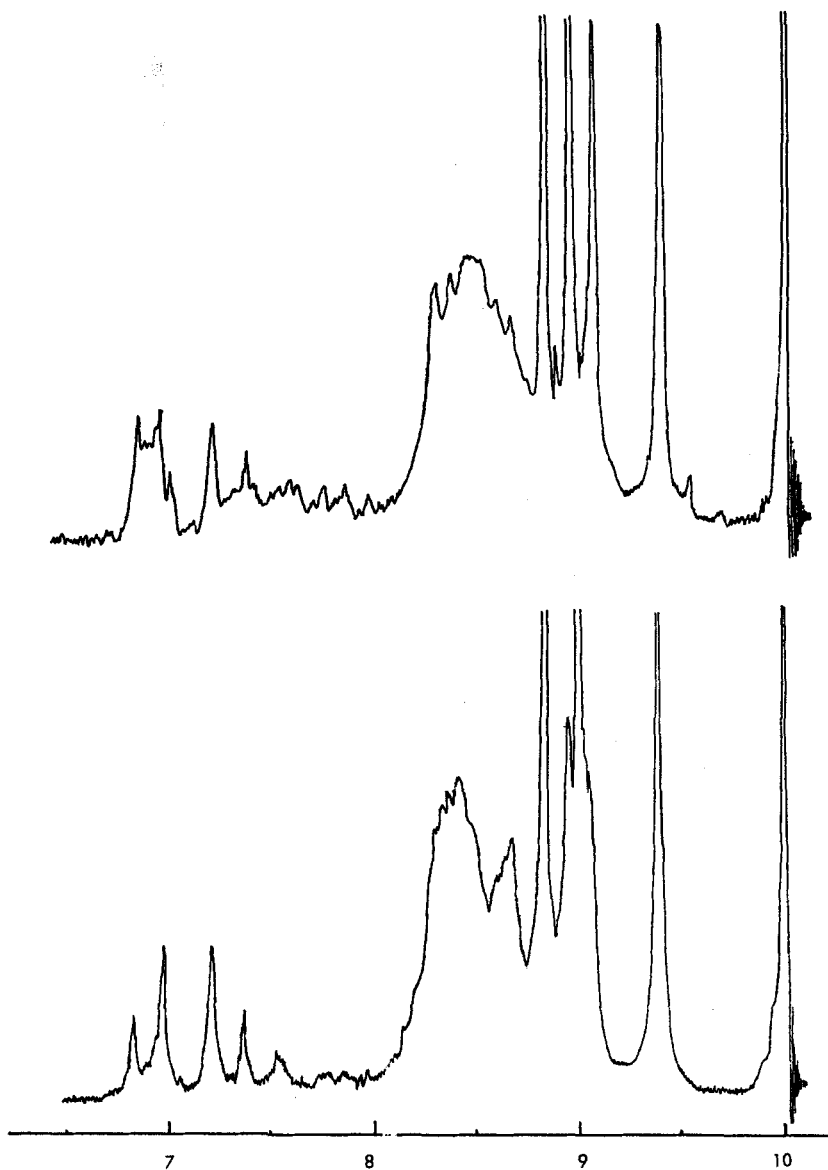
the labelled material (1b) (Fig. 2), it is seen that the AB part of a complex
ABX system at 7.1 $\tau$ in the former compound collapses to a simple AB quartet
in the latter.     These signals have been assigned[4] to the C-1 and C-2
protons and the simple AB spectrum indicates that C-3 of maleopimaric acid
must be deuterated.



(b)   X = $^2$H

(c)   X = $^3$H
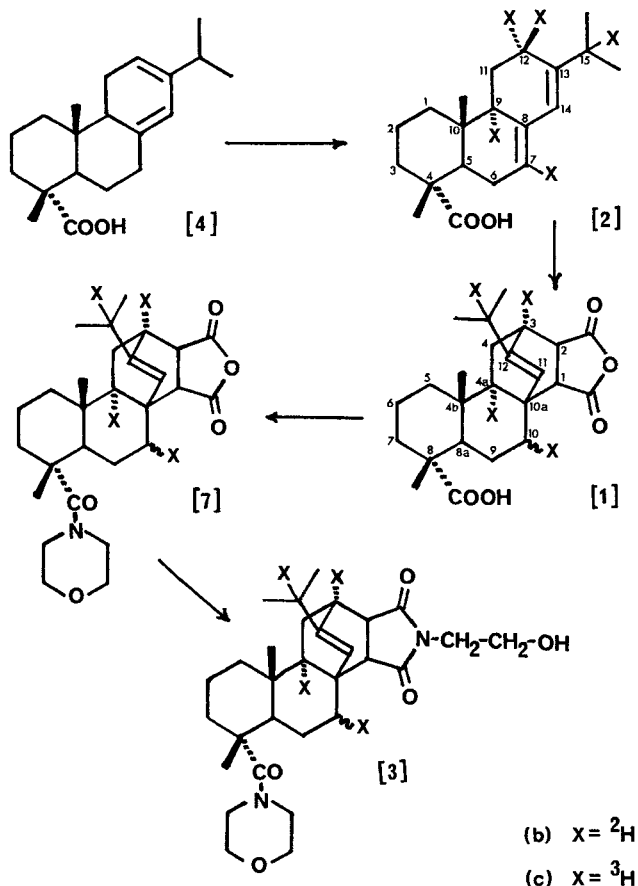
Having assigned the sites of isotopic replacement radioactive labelling
with tritium was undertaken.    Levopimaric acid (4) was treated with
concentrated hydrochloric acid and tritiated water in refluxing dioxan,
resulting in a 31% incorporation of tritium into abietic acid (2c) with a
specific activity of 1.38 mCi/mmole.    The specific activity of maleopimaric
acid (1c) prepared from (2c) was 1.11 mCi/mmole, indicating a 20% loss of
tritium.    When maleopimaric acid (1c) was converted to maleopimaryl
morpholide (7c), the specific activity dropped to 0.98 mCi/mmole, this
apparent loss of activity being explained when (7c) was converted to

N-(2-hydroxyethyl)-maleopimarimidyl morpholide (3c).    This had a specific
activity of 1.00 mCi/mmole, but a high molecular weight impurity not
detected in (7c), was now readily apparent and on purification by column
chromatography the specific activity of RU18492 (3c) was raised to 1.12 mCi/
mmole.    No loss of tritium had thus occurred during the conversion of
maleopimaric acid (1c) to RU18492 (3c).    Furthermore, it was demonstrated
that no loss of radioactivity occurred when (3c) was heated under reflux in
ethanol for 6 hours.

EXPERIMENTAL

Radioactivity was measured on a Nuclear Enterprises NE 6500A
Scintillation Counter, samples being dissolved in 15 ml of a solution of
toluene (1 litre) containing 2,5-diphenyloxazole (4g) and 1,4-bis
[2-(5-phenyloxazolyl)] benzene (100 mg).    Silica gel plates (Merck
Kieselgel 60 F$_{254}$) were used for thin layer chromatography and were
scanned for radioactivity using a Berthold Thin Layer Scanner II.    Nuclear
magnetic resonance spectra were determined on a Perkin Elmer R-12
spectrometer at 60 MHz.    Samples were dissolved in deuterochloroform
containing tetramethylsilane as internal standard.    Melting points were
determined on a Kofler hot stage apparatus and are uncorrected.

[ $^2$H]  - Abietic Acid

a)  Levopimaric acid (4.0g; 13.3 mmole) was dissolved in dioxan
(180 ml) and to the resulting solution was added deuterium oxide (99.8%)
(30 ml) containing concentrated hydrochloric acid (2 ml).    The resulting
solution was stood at room temperature for 24 hours before being poured
into water.    Ether was added and the ethereal layer was separated,
washed twice with water and dried.    Evaporation of the solvent left a
pale yellow gum which was crystallised from ethanol:water, giving colourless
crystals of abietic acid (2.74 g; 9.1 mmole; 68%);   m.p. 172.5-174$^o$.

b)  The experiment was repeated but on this occasion the solution
was heated under reflux for 16 hours.    Similar work up gave abietic acid
(2.82 g;  9.4 mmole;  71%);  m.p. 173-174$^o$.

## [ $^2$H]  – Maleopimaric Acid

a)  [ $^2$H] –Abietic acid (from experiment (a) above) (2.0 g;   6.7 mmole) and maleic anhydride (0.67 g; 6.7 mmole) were ground together to form a fine powder.     The mixture was then heated at 200° for one hour.     After being cooled to ca 80°, the melt was triturated with carbon tetrachloride (5 ml), resulting in the precipitation of the crystalline maleopimaric acid – carbon tetrachloride complex.     The mixture was stood at room temperature for one hour before being filtered, and the solid washed with a small amount of cold carbon tetrachloride.     The yield of the pure maleopimaric acid – carbon tetrachloride complex was 2.85 g.     This material was heated in boiling ethanol (ca 25 ml.) for ten minutes, before water was added.     On cooling crystals of maleopimaric acid (1.32 g; 3.3 mmole;   50%) separated out; m.p. 221-223°.

b)   The experiment was repeated using [ $^2$H] – abietic acid (from experiment (b) above) giving maleopimaric acid (1.26 g; 3.1 mmole; 47%); m.p. 220-223°.

## [ $^3$H]  – Abietic Acid

Levopimaric acid (8g; 26.7 mmole) was added to dioxan (250 ml) containing concentrated hydrochloric acid (2 ml) and tritiated water (0.5 ml; 100 mCi).     The resulting solution was refluxed for 20 hours before being worked up in the manner described earlier.     Crystallisation from ethanol: water gave pure abietic acid (6.83 g; 22.6 mmole; 85%) (31.2 mCi; 1.38 mCi/mmole) m.p. 172-174°.

## [ $^3$H] – Maleopimaric Acid

Abietic acid (6.5g; 21.5 mmole;   1.38 mCi/mmole) and maleic anhydride (2.30g; 23.5 mmole) were reacted together in the manner described earlier giving pure maleopimaric acid (3.88 g; 9.7 mmole; 45%) (10.8 mCi; 1.11 mCi/mmole) m.p. 219-222°.

[$^3$H] – 8$\beta$ – (Morpholino-carbonyl)-4b$\alpha$,8$\alpha$-dimethyl-12-isopropyl-2$\beta$ , 3$\beta$

4,4a$\beta$, 4b$\alpha$, 5,6,7,8a$\beta$, 9,10-dodecahydro [1H] –3,10a – ethenophenanthrene-

1$\alpha$,2$\alpha$ – dicarboxylic acid anhydride (Maleopimaryl morpholide).


To a cooled (0°) solution of maleopimaric acid (3.5 g; 8.8 mmole;

1.11 mCi/mmole) in dry benzene (80 ml), oxalyl chloride (2.5 ml; 3.7 g;

29.1 mmole) was added dropwise with stirring.    After the addition was

complete the reaction mixture was allowed to warm to room temperature.

Evolution of gas ceased after <u>ca</u> 90 minutes and after 4 hours the reaction

mixture was evaporated to dryness <u>in vacuo</u> leaving the crude acid chloride

as a pale yellow crystalline solid.


The acid chloride was redissolved in dry benzene (<u>ca</u> 60 ml) and then

added dropwise to a cooled (0°), stirred solution of morpholine (0.85 ml;

0.85 g; 9.8 mmole) and triethylamine (2.25 ml; 1.75 g; 17.3 mmole) in dry

benzene (20 ml).    After the addition was complete the reaction mixture

was allowed to warm to room temperature, stirring being continued for 20 hours.

Most of the benzene was then removed <u>in vacuo</u> and the residue partitioned

between chloroform and dilute hydrochloric acid.    The chloroform extract was

washed with dilute sodium bicarbonate solution and then with water, and,

after being dried, was evaporated <u>in vacuo</u> to leave a pale yellow gum.

Crystallisation from dichloromethane:ether gave colourless crystals of maleo-

pimaryl morpholide (2.50 g; 5.3 mmole; 60%) (5.18 mCi; 0.98 mCi/mmole)

m.p. 171.5-173°.


[$^3$H] – 8$\beta$ – (Morpholino-carbonyl) – 4b$\alpha$,8$\alpha$-dimethyl – 12-isopropyl – 1$\beta$,

2$\beta$,3$\beta$,4,4a$\beta$,4b,5,6,7,8,8a$\beta$,9,10,10a – tetradecahydro – 3,10a – etheno-

phenanthro [1,2 – c] – 1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione.  [N-(2-

Hydroxyethyl)-maleopimarimidyl morpholide] .


Maleopimaryl morpholide (2.30 g; 4.9 mmole; 0.98 mCi/mmole) was

dissolved in methanol (50 ml) and ethanolamine (0.5 ml; 8.2 mmole) was

added.    The resulting solution was refluxed for 5 hours and then reduced to

a volume of <u>ca</u> 20 ml. <u>in vacuo</u>.    After the addition of water (<u>ca</u> 5 ml),

the solution was cooled, resulting in the deposition of a crystalline solid,

crude N-(2-hydroxyethyl)-maleopimarimidyl morpholide (2.01 g; 3.9 mmole;

80%) (3.94 mCi/mmole) m.p. 194-198°.

This material was chromatographed on alumina (grade V; 220g; 52 x 2.1 cm).    Following elution with benzene:ethyl acetate (9:1) (500 ml), elution with benzene:ethyl acetate (1:1) (1 litre) gave purified material (1.48 g; 2.9 mmole) (3.25 mCi; 1.12 mCi/mmole) m.p. 198-201°. Recrystallisation from methanol gave pure N-(2-hydroxyethyl)-maleopimarimidyl morpholide (1.22 g; 2.38 mmole) (2.66 mCi; 1.12 mCi/mmole) m.p. 199.5 – 201.5°.

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