SYNTHESIS OF METHYL TRANS-(10-^{14}C)-RETINOATE AND TRANS-(10-^{14}C)-RETINOL

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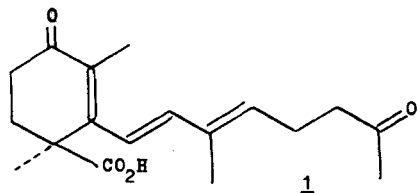
SUMMARY

The preparation of the title compounds, using β-ionone, is accomplished in fair overall radiochemical yield from (2-^{14}C) bromacetic acid, in 7 and 8 steps respectively. The sequence utilises ylid reactions of the phosphonoacetate derived from bromacetic ester, and is also capable of the introduction of label at C-14.

MATERIALS AND METHODS

In connection with continuing work on the biosynthesis of trisporic acid B (1) and related compounds [1] in certain fungi, we required to synthesise trans 6-methyl-8(2,6,6-trimethyl-1-cyclohexen-1-yl)-3,5,7-octatrien-2-one (5, hereafter referred to as trans C_{18}-ketone) and trans 3,7-dimethyl-9(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol (7, hereafter referred to as trans retinol), suitably labelled with ^{14}C.



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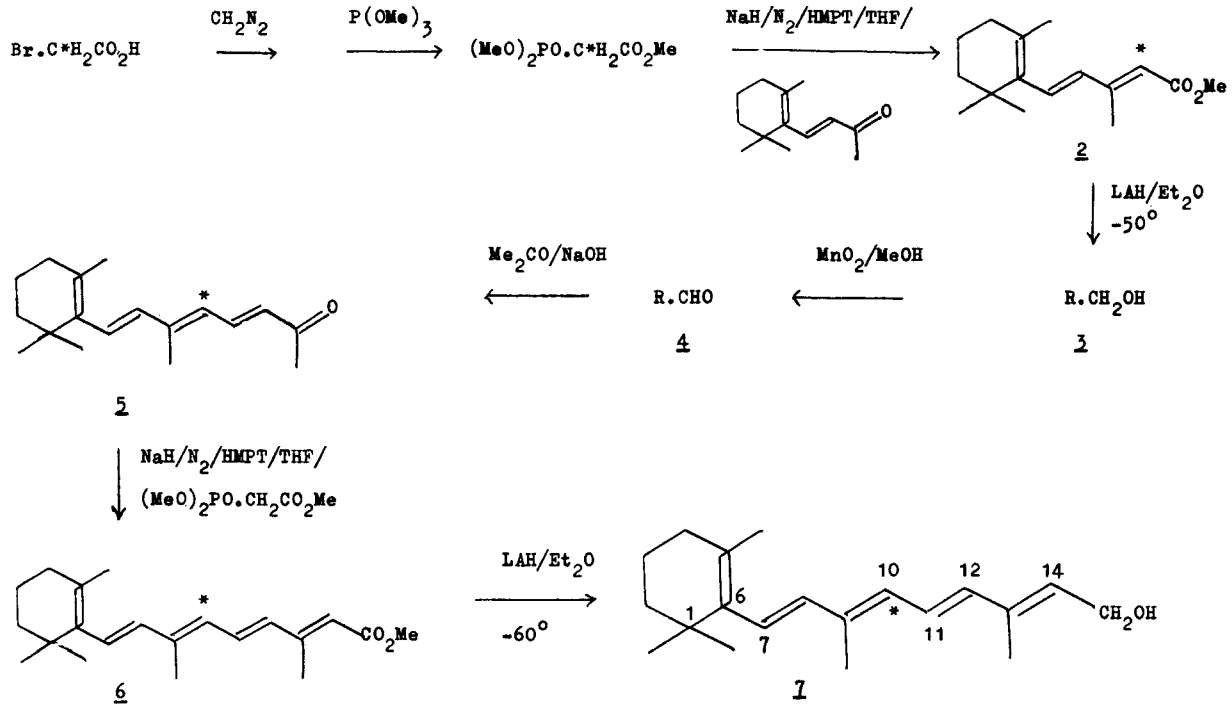
Br.C*H2CO2H
CH2N2
P(OMe)3
(MeO)2PO.C*H2CO2Me
NaH/N2/HMPT/THF/
CO2Me
2
LAH/Et2O
-50°
R.CH2OH
3
MnO2/MeOH
R.CHO
4
Me2CO/NaOH
5
NaH/N2/HMPT/THF/
(MeO)2PO.CH2CO2Me
6
LAH/Et2O
-60°
CH2OH
7
KEY: LAH = Lithium aluminium hydride
THF = Tetrahydrofuran
HMPT = Hexamethylphosphoric triamide
= R

The synthesis and labelling of retinol and related compounds has been reviewed.[2,3] Retinols labelled by ^{14}C at positions 6,7; 14; 15; and by ^{3}H at 11, 12 (IUPAC numbering, as shown in 7) have been reported.[3] We now describe the preparation of trans-[10-^{14}C]-retinol via trans-[10-^{14}C]-C_{18} ketone and methyl trans-[10-^{14}C]-retinoate, in 20% yield from [2-^{14}C]-bromacetic acid by a method also adaptable to the synthesis of trans-[14-^{14}C]-retinol in higher yield. The synthesis utilizes the phosphonate ester modification of the Wittig reaction,[4] an approach adopted by Huisman and co-workers in the synthesis of 4-thiaretinol.[5a] The sequence is depicted in the Scheme.

The route is attractive since stereoisomers with trans double bonds greatly predominate in the ylid reactions, and all the steps with the exception of the aldol condensation 4 → 5 proceed in good yields of purified compounds. The yields in the reactions, with phosphonate carbanions are critically dependent on

a) the use of hexamethylphosphoric triamide (HMPT) as co-solvent to increase their nucleophilicity[6] and

b) the use of an excess of trimethylphosphonoacetate relative to sodium hydride and substrate ketone.

The yields in the reduction reactions are critically dependent upon temperature.

Experimental

Merck Silica Gel t.l.c. plates (250μ) were used to monitor purity. Preparative separations were performed on Merck Silica Gel F_{254} plates (2mm). G.l.c. was carried out on a Perkin-Elmer F-11 instrument. Infra-red (IR) spectra were measured on a Perkin-Elmer 257 Spectrometer and ultra-violet (UV) spectra on a Unicam SP-800 Spectrometer.

Reactions resulting in the formation of compounds 2 - 7 were conveniently followed by withdrawing aliquots and examining UV spectra; those of final products isolated were in all cases in complete agreement with the UV spectral data plotted and tabulated by Huisman.[5a]

Radioactivities were determined by liquid scintillation counting on a Packard Tricarb Liquid Scintillation Counter Model 3320.

Reactions performed under nitrogen were, unless otherwise specified, conducted by flushing the flask, containing a magnetic stirrer bead if required, with dry nitrogen and sealing with a vaccine cap pierced by a syringe needle connected to a static nitrogen head. All necessary transfers and additions of solutions were made using syringes.

Solvents were removed on a rotary evaporator at room temperature in all cases.

Purification of reagents and solvents

Methyl bromoacetate was purified by filtering from sodium carbonate and distilling, collecting the fraction boiling at 146-8°/770 mm.

Trimethyl phosphite was purified by reflux over, and distillation from, sodium hydride under dry nitrogen. The fraction boiling at 111-2°/770 mm was collected.

β-Ionone was purified by distillation at reduced pressure under nitrogen, collecting the fraction boiling at 134-6°/12 mm.

THF was purified by reflux over, and distillation from, LAH under dry nitrogen. The fraction boiling at 65-7°/770 mm was collected and stored in the dark under nitrogen over 3A Molecular Sieve in a flask stoppered with a vaccine cap. Diethyl ether, b.p. 34-5°/770 mm was purified and stored in the same way.

HMPT, b.p. 232°/770 mm was purified by distillation under nitrogen and storage over 13 X Molecular Sieve under nitrogen in a vaccine cap-stoppered flask.

Methyl [2-^{14}C]-bromoacetate

An ampoule of [2-^{14}C]-bromacetic acid (250μCi, specific activity 55 mCi/mmole, 98% pure, supplied by the Radiochemical Centre, Amersham) was cooled in dry ice/acetone, opened and treated at once with excess diazomethane in ether. The solution was quantitatively transferred to methyl bromoacetate (660 mg, 4.31 mmole) contained in a 5 ml stoppered flask and the mixture allowed to stand 10 mins, after which a yellow colour was still apparent.

The ether was removed.

[2-^{14}C]-Trimethylphosphonoacetate

Trimethyl phosphite (0.9 ml) was added to the methyl [2-^{14}C]-bromoacetate, the mixture refluxed under dry nitrogen in a 120° oil bath for 90 mins, cooled and transferred, washing with pure trimethyl phosphite, to a 5 ml Quickfit micro-distillation apparatus fitted with a dry nitrogen bleed for vacuum distillation. The pressure was slowly reduced to 15 mm and, when the liquid had de-gassed, the flask warmed in a 100° oil bath. Trimethyl phosphite distilled at 70°. The temperature of the bath was raised to 160°, and the [2-^{14}C]-trimethylphosphonoacetate distilled at 130°/15 mm. Yield 636 mg (81%). This was stored under nitrogen at -5°. ν_{max}(film): 1738, 1270, 1052, 1028 cm^{-1}.

Methyl trans-[10-^{14}C]-β-ionylidene acetate (2)

A 50% dispersion of sodium hydride in oil (120 mg, 2.50 mmole) was weighed into a 10 ml flask containing a magnetic stirrer bead. The flask was adapted to carry out reactions under nitrogen. [2-^{14}C]-trimethylphosphonoacetate (636 mg, 3.49 mmole) was added to the stirred flask contents using THF (2 x 2 ml) to carry and wash. HMPT (2 ml) was added and the mixture stirred in the dark for 1 hour, by which time it was clear and cool.

β-Ionone (481 mg, 2.51 mmole) was added using THF (2 x 0.5 ml) to carry and wash. The stirred reaction was left in the dark for 65 hr, after which it was partitioned between 40-60° petrol (5 ml) and water (5 ml). The organic phase was washed with water (2 x 5 ml), dried (Na_2CO_3), filtered and evaporated in a 10 ml flask to yield 608 mg (70%, relative to trimethylphosphonoacetate). T.l.c. (60-80° petrol/acetone 9/1), showed a faint trace of β-ionone, R_f = 0.49, as a purple spot, and methyl trans-β-ionylidene acetate, R_f = 0.65, as a yellow spot turning red on warming briefly, after visualisation with conc. H_2SO_4.

ν_{max} (film): 1710, 1605, 1228, 1148, 1040, 965 cm^{-1}.

Trans-[10-^{14}C]-β-ionylidene ethanol (3)

LAH (3 *g*) was stirred in the cold under nitrogen with diethyl ether (100 ml) for 3 hr, the suspension allowed to settle, a supernatant aliquot

withdrawn and spun down in a centrifuge tube flushed with nitrogen and closed with a vaccine cap. The resulting supernatant contained 3.5 mg LAH/100 µl, as determined by decomposition of 200 µl in 20% ethanolic ether in a gas burette.

Methyl trans-[10-^{14}C]-β-ionylidene acetate (608 mg, 2.44 mmole) was dissolved in diethyl ether (3 ml) in a 10 ml flask equipped with stirrer bead and set up for work under a nitrogen atmosphere. The flask was enclosed in tin foil to shield from light, cooled in a dry ice/acetone bath at -50° and an ethereal solution of LAH (2.5 ml, prepared as above) added with stirring.

After 45 mins, during which time the temperature rose to, and was maintained at, 0°, the reaction was again cooled to -50° and Analar methanol (3 ml) added slowly with stirring. The resulting mixture was allowed to come to 0° and partitioned between 40-60° petrol (8 ml) and 1N H_2SO_4 (3 ml), both pre-cooled to 0°. The organic phase was washed with iced water (2 x 5 ml), dried (Na_2CO_3), filtered and evaporated to yield 505 mg (94%). This was kept at -5°, dissolved in a little methanol, and used as soon as possible.

T.l.c. (benzene/ethyl acetate 9/1) showed only trans-β-ionylidene ethanol, R_f = 0.36, as a green spot on spraying with conc. H_2SO_4 and warming briefly.

ν_{max} (film): 3300, 1630, 1610, 1010, 970 cm^{-1}.

Trans-[10-^{14}C]-β-ionylidene acetaldehyde (4)

Active manganese dioxide was prepared by a modification of Morton's procedure.(7) $MnSO_4.4H_2O$ (167 g, 0.75 mole) in deionised water (220 ml) was heated to 70° with stirring. A solution of $KMnO_4$ (82 g, 0.52 mole) in deionised water (1300 ml), stirred and maintained at 70°, was added to the manganous sulphate solution over 35 mins without allowing the temperature to fall. Stirring was continued a further 5 mins after the addition was complete and the mixture allowed to settle for 30 mins, maintaining the temperature. The acidic supernatant was decanted and replaced with deionised water (1 l) preheated to 75°. The mixture was again stirred for several minutes, and allowed to settle and cool for 15 mins. The supernatant was decanted and the washing procedure repeated with hot water until the washings were neutral. A final washing with cold water

gave 114 g after filtration, drying in a 75° oven, powdering and desiccation over KOH. This material is pyrophoric when dampened (not wetted) with methanol or acetone.

Active manganese dioxide (3 g) was added (CARE!) in small portions with stirring to Analar methanol (50 ml) cooled to 0°. Trans-[10-^{14}C]-β-ionylidene ethanol (505 mg, 2.30 mmoles) in methanol (5 ml) was added dropwise to the vigorously-stirred suspension at room temperature and the stirred reaction left in the dark for 4 hr.

The reaction mixture was filtered with gentle suction through a No. 3 sinter, and the MnO_2 residue washed with methanol until the washings were clear to UV. The filtrate and washings were evaporated to low bulk, centrifuged, and the supernatant evaporated to dryness on a rotary evaporator. This gave 486 mg (97%) of product which was used at once. T.l.c. (60-80° petrol/acetone 9/1) showed only trans-β-ionylidene acetaldehyde, R_f = 0.57, as a purple spot on spraying with conc. H_2SO_4 and warming. The compound showed a retention time of 1 min 42 sec at 200° on g.l.c. using a 1 metre column of 8% polyethylene glycol adipate and a carrier flowrate of 50 ml/min.

ν_{max}(film): 1664, 1605 cm^{-1}.

Trans-[10-^{14}C]-C_{18} ketone (5)

Trans-[10-^{14}C]-β-ionylidene acetaldehyde (486 mg, 2.23 mmole) was dissolved in Analar acetone (7 ml) in a flask equipped with a magnetic stirrer bead. 1.0N NaOH (0.7 ml) was added, the flask flushed with nitrogen and the reaction stirred in the dark for 200 mins. Water (8 ml) and 60-80° petrol (15 ml) were added. The organic phase was separated after shaking, washed with water (2 x 5 ml), dried (Na_2CO_3), filtered and evaporated to yield 518 mg of crude product. This was purified by chromatography in subdued light, using Spence Activated Alumina Type 'H' 100-200 mesh (24 g), eluting with dichloromethane. A bright yellow band separated from a faster-running deep orange component. (The mass spectrum of the latter suggested it arose by aldol condensation between starting material and product). The yellow band was collected in 3 ml fractions, which were assayed by UV and t.l.c. (60-80° petrol/acetone 9/1). The trans-C_{18} ketone showed as a red spot which fades, R_f = 0.43, on spraying with conc. H_2SO_4. Pure fractions were combined to give 313 mg (54%) on workup. The specific

activity of this material was 57.0 μCi/mmole.

The semicarbazone was prepared and recrystallised,[8] affording material m.p. 191° (Kofler hot stage)(Found: C, 72.3; H, 9.2; N, 13.3. Calc. for $C_{19}H_{29}N_3O$: C, 72.4; H, 9.2; N, 13.3%).

The purity of the ketone was confirmed by g.l.c. It showed a retention time of 8 mins 30 sec using conditions identical to those already described for trans ionylidene acetaldehyde.

The compound was dissolved in 40-60° petrol and stored in the dark under nitrogen at -5°. It was used as soon as possible.

ν_{max} (film): 1660, 1588, 1569, 1249, 960 cm^{-1}.

Methyl trans-[10-^{14}C]-retinoate (6)

A 50% dispersion of sodium hydride in oil (21 mg, 440 μmole) was suspended in THF (1.0 ml) in a 5 ml flask containing a magnetic stirrer bead and adapted to carry out reactions under nitrogen. Trimethylphosphonoacetate (72 μl, approx. 500 μmole) and HMPT (0.4 ml) were added and the reaction stirred till cool and clear. The reagent was added to a similar flask containing trans-[10-^{14}C]-C_{18} ketone (46 mg,178 μmole) and THF (0.4 ml). The reaction was stirred at room temperature in the dark for 64 hr. 40-60° petrol (5 ml) and water (5 ml) were added and the mixture shaken. The organic phase was separated and washed with water (2 x 3 ml), dried (Na_2CO_3), filtered and evaporated to yield 46 mg of material with a UV and t.l.c. indicative of satisfactory purity. However, as the retinol prepared by exceptionally clean reaction in the next stage is labile to handling, a precautionary purification of the methyl trans-retinoate was nevertheless undertaken. Preparative t.l.c. (60-80° petrol/acetone 9/1) in the dark in a tank flushed with nitrogen gave 40.8 mg (73%), R_f = 0.70. The yellow material appears as a magenta spot on spraying with conc. H_2SO_4.

ν_{max}(film): 1709, 1605, 1582, 1234, 1149 cm^{-1}.

Trans-[10-^{14}C]-retinol (7)

Methyl trans-[10-^{14}C]-retinoate (40.8 mg, 130 μmole) was dissolved in diethyl ether (1 ml) in a 5 ml flask containing a magnetic stirrer bead and set

up for work under a nitrogen atmosphere. The flask was shielded from light in tin foil and cooled in a dry ice/acetone bath at -60°. The solution of LAH in ether (300 µl, see preparation of trans-β-ionylidene ethanol) was added with stirring, keeping the temperature below -30° throughout. After 20 mins, the solution was cooled again to -60° and Analar methanol (0.5 ml) added dropwise with stirring. The resulting solution was partitioned between 40-60° petrol (8 ml) and 1N H_2SO_4 (2 ml), precooled to 0°. The organic phase was speedily washed with ice water (2 x 2 ml), dried (Na_2CO_3) and evaporated to yield 37.2 mg (100%) trans-[10-^{14}C]-retinol. The compound was pure by spectral and t.l.c. criteria, showing one lilac spot, R_f = 0.20 (benzene/ethyl acetate 95/5) on spraying with conc. H_2SO_4 and warming. Spectral data were in complete agreement with published values.[5a, 5b]

The compound had specific activity 57.0 µCi/mmole confirmed (20% overall yield from bromacetic acid). It is not stable, and was used immediately.

In preliminary runs using "cold" C_{18} ketone on a 1 mmole scale and increasing all quantities reported in the preparation of methyl trans-[10-^{14}C]-retinoate five-fold, a 37% yield (relative to excess phosphonate) of pure product was obtained. In principle, therefore, the reaction is capable of providing methyl trans-[14-^{14}C]-retinoate in approximately 30% yield from [2-^{14}C]bromacetic acid. This would represent a 50% improvement on the previously reported procedure.[9]

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