SYNTHESIS OF DIMETHYL 5, 5, 6, 6 ($^2H_4$)-2-OXOHEPTYLPHOSPHONATE, A DEUTERIUM LABELLED PROSTAGLANDIN INTERMEDIATE

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SUMMARY

The synthesis of the specifically labelled versatile prostaglandin intermediate 1b is described which involves the preparation of 2,2,3,3 ($^2H_4$)butyliodide 6b via homogenous catalytic deuteration of the acetylene 4. Examples for the preparation of prostaglandins and extensive spectroscopic data are included.

Key Words: catalytic deuteration, prostaglandin

All known prostanoids including numerous metabolites possess a 8-carbon ω-side chain which can be readily introduced in the course of the Corey bicyclic lactone route by an Emmons-Horner-Wittig reaction of the phosphonate 1a (1,2), to give the versatile intermediate 2a (3-6). Thus isotopically labelled dimethyl 2- oxoheptylphosphonate 1b could serve as a building block for a variety of labelled prostanoids, which are of high interest in bioscience to be used as internal standards in the GC/MS determination of prostanoids (7). We wish to report a rapid and economic synthesis of the deuterium labelled prostaglandin intermediate 1b.

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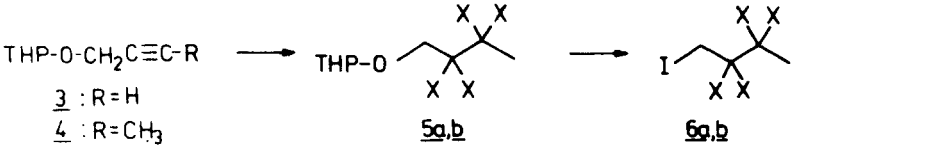
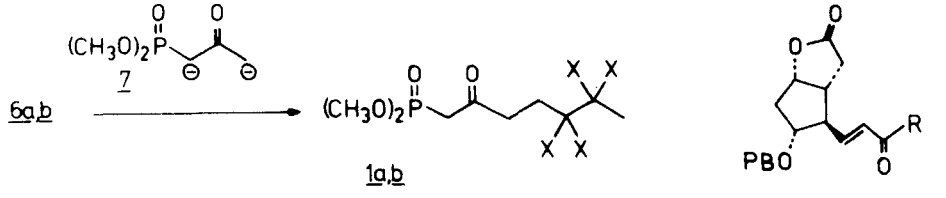
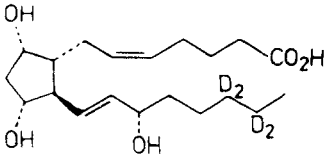
RESULTS

The THP- ether 3, which is readily obtained from dihydropyran and propargyl alcohol (8), is converted into the lithium acetylide (9) and subsequently C-alkylated with methyl iodide to the THP-ether 4. This acetylene may be saturated upon homogenous catalytic deuteration ($(Ph_3P)_3$ RhCl) to give 5b. Following the procedure previously described for other ethers (10, 11), the labelled butyl THP-ether 5b is cleaved ($H_3PO_4/KI/P_2O_5$) to yield the ($^2H_4$) butyliodide 6b. According to the procedure of Grieco (12), terminal alkylation of the dianion 7 with 6b affords the desired dimethyl 5,5,6,6 ($^2H_4$)-2-oxoheptylphosphonate 1b, from which the common intermediate 2b of various prostaglandins (e.g. 8, d_4- $PGF_{2\alpha}$), labelled in the positions C-18 and C-19 (13) is obtained.







THP=2-tetrahydropyranyl

PB =4-C_6H_5-C_6H_4-C(=O)-

a : X =H

b : X =D

DISCUSSION

The crucial step in the synthesis of labelled internal standards, used in the isotope dilution technique, is the introduction of deuterium. As the amount of unlabelled compound (d_o) determines the detection limit of the analytical GC/MS measurement, the isotopic purity of the internal standard should be as high as possible. First attempts to prepare 5b by heterogenous catalytic deuteration (Pd/C and Rh/C) led to products which showed extensive scrambling due to allenic rearrangements, detectable by ^{13}C-NMR and mass spectrometry (d_1-d_7, vide infra). Rearrangements occur too, when unprotected propargylalcohol is hydrogenated (14). Absolutely no scrambling could be observed, when protected 4 is deuterated homogenously using Wilkinson's catalyst under the conditions described for steroids (15, 16). Measuring the abundant $(M-H)^{+\cdot}$ fragment ion of 5b, selected ion monitoring (MS/SIM) proved that unlabelled 5a was undetectable ($d_o < 0.05\%$). Similar results are obtained within the detection limit with 2b ($d_o < 0.2\%$) and the prostaglandin 8 ($d_o < 0.1\%$).

As prostaglandins are metabolized predominantly by ß-oxidation of the upper (α) side chain, isotope isomers labelled in the lower (ω) side chain are suitable substrates for further chemical and biochemical conversions (17).

EXPERIMENTAL SECTION

General. Unless otherwise noted all reagents were used as purchased and were Reagent Grade where available: lithium amide, alumina (Al_2O_3-90, 'aktiv basisch'), n-butyllithium (1.6m in n-hexane), methyl iodide (E.Merck); tris (triphenylphosphine) rhodium (I)-chloride (Alfa/ Ventron), molecular deuterium (> 99.5%, Linde), dimethyl 2-oxopropylphosphonate, dimethyl 2-oxoheptylposphonate (Aldrich / EGA), sodium hydride (55-60% in mineral oil, Fluka). Melting points were determined on a 'Electrothermal' melting point apparatus and are uncorrected. IR spectra were taken on a Perkin- Elmer spectrometer, Model 283, only characteristic or intense absorptions ($\tilde{\nu}$, cm^{-1}) are given. The NMR spectra were obtained at 80 MHz (^{1}H) or 20.1 MHz (^{13}C,

complete proton decoupled) on a Bruker WP 80-DS spectrometer (39°C) using $CDCl_3$ as solvent and tetramethylsilane as internal standard; all shifts are given in ppm (δ-values). Mass spectra were recorded on a Hewlett- Packard spectrometer, Model 5985 (IE 11 eV and 70 eV; m/z as indicated). Thin- layer chromatography (TLC) was conducted using E.Merck 'Fertigplatten', precoated with silica gel 60-F_{254}. The TLC spots were visualized first by UV light then either by spraying with alcoholic phosphomolybdic acid (3.5%) or 2.4-dinitrophenylhydrazine (5% in 70: 20: 10, $EtOH/H_2O/HClO_4$) followed by heating. Unless otherwise noted, column chromatography utilized pre-packed columns of neutral silica gel ('Lobar', E.Merck).

2-(Prop- 2'-yne-1'-yloxy) tetrahydropyran, 3 was prepared as described (8), b.p. 69-71°C/17mm (84-93%).

2- (But- 2'-yne-1'-yloxy) tetrahydropyran, 4.

To a stirred solution of 3 (91g, 0.65 mol) in a mixture of dry hexamethylphosphoramide (20ml) and dry tetrahydrofuran (500ml) was added 18.6g (0.81mol) of $LiNH_2$ under an atmosphere of argon. The resultant suspension was stirred for 30 min at 40°C, and then refluxed for 4 hr. After the apparatus was flushed with argon to remove excess ammonia, the mixture was cooled with an ice bath and methyl iodide (100 ml, 1.6mol) was added dropwise over a 2 hr period. The reaction mixture was refluxed for 3 hr and then cooled to ambient temperature. After the addition of diethyl ether (400 ml) the precipitated solid was removed by filtration and the filtrate was concentrated. The dark brown residue was partitioned between water (1.5l) and n-hexane (300ml). The aqueous layer was extracted with two 300 ml portions of n-hexane. The combined organic extracts were washed with water (4x400 ml), dried with K_2CO_3, and evaporated under reduced pressure. Distillation gave pure 4 as a colourless liquid in 71-82% yield (0.1 - 0.65 mol runs). b.p. 99-101°C/17 mm, n_D^{20}=1.4668

found C 70.01%, H 8.95%; calc. for $C_9H_{14}O_2$ (154. 2) C 70.10%, H 9.15%.

TLC (EtOAc/n- Hexane, 1:4) :R_f0.70.

^{1}H-NMR:1.4- 1.8 (6H, m, H-3,4,5), 1.85 (3H, t, J=2.2 Hz, H-4'), 3.3- 4.05 (2H, m, H-6), 4.22 (2H, tripl. of dupl., J= 2.4/ 1.9 Hz, H-1'), 4.79 (1H, broad t,J~4Hz, H-2).

^{13}C-NMR:3.6 (C-4'), 82.2 (C-3'), 75.2 (C-2'), 54.7(C-1'), 62.1 (C-6), 25.5 (C-5), 19.2 (C-4), 30.4 (C-3), 97.0 (C-2). The assignment of the resonances is based on the data given for THP- ethers (18) and propargyl alcohols (19).

IR (film):intense absorptions at 2950, 1115, 1022.

Using n-butyl lithium (O°C) instead of $LiNH_2$ and equimolar amounts of methyl iodide (O°C, 24hr), the reaction gave 4 in yields of 54- 60%.

2-(2',2',3',3',($^2H_4$)- But-1'-yloxy) tetrahydropyran, 5b.

4g (4.3 mmol) of tris (triphenylphosphine) rhodium (I)-chloride was suspended under an atmosphere of deuterium gas in 70 ml of ethyl acetate. The mixture was stirred for 1 hr at room temperature, and then 41.5g (0.27 mol) of 4 was added in 3-5 portions over ca. 36 hr in order to maintain a deuterium uptake of approximately 400-500ml D_2/hr. The reduction was allowed to proceed for ca. 48hr, after which deuterium gas uptake had ceased. The apparatus was then flushed with argon, 500ml of n-pentane was added, and stirring was continued for 30min. After filtration the solution was concentrated on a rotary evaporator and chromatographed on alumina using n-pentane as solvent. Removal of the solvent left a faint yellow liquid (39.5g, 90.4%) which was sufficiently pure for the next step. In several runs yields of crude product were 90% to quantitative. An analytical sample was distilled to give a colourless liquid, b.p. 71.5-72°C/ 17 mm, n_D^{20}=1.4320

found C 66.66%, H 8.67%, D 4.94%; calc. for $C_9H_{14}D_4O_2$ (162.3) C 66.62%, H 8.69%, D 4.96%.

IR (film):2950, 2200, 2120, 1125, 1039

^{1}H-NMR:0.97 (3H, quint., J=0.9 Hz, H-4'), 1.4- 1.9 (6H, m, H-3, 4, 5), 3.2 - 4.05 (4H, m, H- 6, 1'), 4.57 (1H, broad t, J~4 Hz, H- 2).

^{13}C-NMR:13.7 (C-4'), 18.6 (C-3'. quint., J= 19Hz), 31.0(C-2', quint., J= 19Hz), 67.4 (C-1'), 62.4 (C-6), 25.7 (C-5), 19.8 (C-4), 31.0 (C-3), 99.1 (C-2).

MS: (GC, 11 eV): 85 (100%, THP- fragment), 115 (13.8%), 161 (26.4%, $M^{+\cdot}$- H), 162 (6.2%, $M^{+\cdot}$). In the SIM mode (11 eV) the ratio of the $(M-H)^{+\cdot}$ peaks (d_0: 157.2; d_4: 161.2) gave a content of unlabelled 5a of $\lesssim$0.05%.

When 4 (4mmole) was deuterated using 10% Rh/C or 5% Pd/C (200 mg or 100 mg, 3ml EtOAc, 1 hr, 20°C) as heterogenous catalyst, 5b could be isolated in quantitative yield. Significant differences to 5b, obtained from homogenous deuteration, were observed in the ^{13}C-NMR spectra and MS. The C-1' carbon (O-CH_2) gave a singlet at 67.4 ppm of reduced intensity and a superimposed multiplet. The CH_3- resonance (C-4', 13.7 ppm) was a multiplett too with a very weak singlet. These findings indicate deuterium incorporation into the CH_2- and CH_3- groups adjacent to the acetylenic bond due to allenic rearrangements. Extensive scrambling exhibits the mass spectrum of this compd. in the region of the $(M-H)^{+\cdot}$ fragment ion: d_0 (n.d.), d_1 (2%), d_2(13%), d_3(32%), d_4(35%), d_5(14%), d_6(3%), d_7(0.1%).

2-(But-1'-yloxy) tetrahydropyran, 5a, was prepared from dihydropyran and n-butanol; ^{13}C-NMR: with the exception of the unlabelled carbon atoms (C-2': 32.1; C-3': 19.6), the spectrum showed essentially the same shift positions as 5b.

2,2,3,3 ($^2H_4$)-1-Iodobutane, 6b.

A cooled mixture (0°C) of P_2O_5 (33g), potassium iodide (159 g), and H_3PO_4 (123 g, 85%) was stirred for 30 min. To this solution 31.3 g (0.19 mol) of 5b was added

dropwise over 1 hr. After the addition was complete, the black and viscous mixture was refluxed (pot temperature 125°C) for 2 hr. After cooling to room temperature, the reaction mixture was thoroughly extracted with n-pentane. The combined extracts were washed with water, dried over Na_2SO_4, and concentrated on a rotary evaporator (<25°C). Further purification of crude 6b was accomplished by filtration through alumina, elution with n-pentane, and distillation. In several runs pure 6b was isolated in yields of 54-63%, b.p. 126°C, n_D20 = 1.4980.

found C 25.48%, H 2.61%,D 4.16%, I 67.32%; calc. for $C_4H_5D_4I$ (188.0)

C 25,55%, H 2.68%, D 4.28%, I 67.48%.

IR (film): 2960, 2195, 2116, 1455, 1194.

^{1}H-NMR:0.91 (3H, quint., J=1 Hz, H-4), 3.18 (2H, quint.,J=1 Hz, H-1); unlabelled 6a showed resonances at 0.92 (3H, tripl., J= 7.3 Hz, H-4), 1.2-2.0 (4H, m, H- 2, 3), 3.20 (2H, tripl., J= 6.8 Hz, H-1).

^{13}C-NMR:6.4 (C-1), 34.7 (quint., J= 19.4 Hz, C-2), 22.7 (quint., J= 19.4 Hz, C-3), 12.7 (C-4). In unlabelled 6a the methylene carbons gave singlets at 35.5 (C-2) and 23.7 (C-3) (see (20, 21)).

Dimethyl 5, 5, 6, 6- ($^2H_4$) 2- oxoheptylphosphonate, 1b.

Sodium hydride (12.2g, 0.29mol, 55-60%) was placed under argon in a three-necked flask equipped with an overhead stirrer. Anhydrous THF (500ml) was then added followed by the slow addition (1 hr, 25°c) of dimethyl 2-oxopropylphosphonate 7 (43.1g, 0.26mol). After stirring for 1 hr at 25°C, the reaction was cooled to 0°C, and 172 ml (0.27mol) of 1.6m n-butyllithium in n-hexane was added dropwise over a 90min period. The resultant clear solution of the dianion of 7 was stirred for 30min and the labelled n-butyliodide 6b (57.5g, 0.3mol) was added over 1 hr(0°C,). After 18hr (0°C) the solution was neutralized with 5% aqueous HCl and saturated with solid NaCl. The organic phase was separated, concentrated under vakuum on a rotary evaporator, and

dissolved in 300ml of CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (4x100ml). The combined organic phases were washed with water (2x100ml), dried over Na_2SO_4, and concentrated to give 44g of viscous red-brown oil. The ^{1}H-NMR spectrum showed a molar ratio of 75-80% of 1b and 20-25% of 7. Distillation of the crude mixture affords 24-27g (40-45%) of 1b (b.p.78-82°C/0.01mm) sufficiently pure (92-98%) for further reactions though some residual starting material (dimethyl 2-oxopropylphosphonate 7, 2-8%) was still present. Both the phosphonates may be distinguished and further purified by TLC and column chromatography on silica gel. TLC (EtOAc/5% MeOH): R_f 0.26 (7), and R_f 0.47 (1), yellow spots with iodine vapour.

An analytical sample of 1b gave the following data:

found C 47.88%, H 6.68%, D 3.56%, calc. for $C_9H_{15}D_4O_4P$ (226.2) C 47.78%, H 6.68%, D 3.56%. n_D^{20}=1.4423.

IR (film): 2960, 2190, 2110, 1717, 1260, 1030.

^{1}H-NMR (22): 0.87 (3H, broad singl., H-7), 1.57 (2H, broad tripl., J= 7.3 HZ, H-4), 2.61 (2H, tripl., J= 7.3 Hz, H- 3), 3.08 (2H, dupl., J= 22.9 Hz, H-1), 3.79 (6H, dupl., J= 11.2 Hz, OCH_3).

^{13}C-NMR (23): 41.4 (dupl., J= 129 Hz, C- 1), 202.3 (dupl., J= 6.1 Hz, C- 2), 44.2 (C-3), 22.9 (C-4), 30.2 (quint., J= 19.5 Hz, C-5), 21.4 (quint., J= 19.5 Hz, C- 6),13.6 (C-7), 53.1 (dupl., J= 6.1 Hz, OCH_3).

MS (70 eV, DI): 79 (42%), 94 (59%), 109 (74%), 124 (100%), 151 (83%), 167 (32%), 168 (12%), 179 (33%), 180 (13%), 195 (5%), 226 ($M^{+\cdot}$, 3.5%).

A slightly different work-up (neutralization with acetic acid, filtration of the crude product through silica followed by elution with methanol) gave a polymeric product upon distillation and only traces of the desired phosphonate 1b could be isolated.

(d1)- 5α- Hydroxy- 2ß (6', 6', 7', 7' ($^2H_4$)- 3'- oxo- trans- 1'- octenyl)- 3α - (4- phenybenzoyloxy) cyclopentane- 1α - acetic acid ϑ- lactone, 2b.

The enone 2b was prepared from racemic Corey's lactone and 1b in dry DME according to published procedures (4,5). Chromatography of the crude product(SiO_2, - CH_2Cl_2/EtOAc, 10: 1) and recrystallization from EtOAc/ n- hexane afforded pure 2b, m.p. 126°C, white fibrous crystals.

TLC: R_f 0.36 (EtOAc/ n- hexane, 2:3), R_f 0.50 (CH_2 Cl_2/ EtOAc, 10: 1).

Found C 74.54%, H 5.72%, D 1.76%; calc. for $C_{28}H_{26}D_4O_5$(450.5) C 74.64%, H 5.82%, D 1.79%.

IR (KBr): 1770, 1760, 1720, 1693, 1630, 1610, 1277, 1172, 1114, 740.

^{1}H-NMR (6, 13): 0.86 (3H, broad singl., H- 20), 1.58 (2H, tripl., J~7 Hz, H- 17), 2.1-3.1 (8H, m), 5.13 (1H, m, H- 9), 5.37 (1H, quadr., J~5 Hz, H- 11), 6.24 (1H, dupl., J= 15.7 Hz, H- 14), 6.73 (1H, dupl. of dupl., J= 7.1 Hz, 15.7 Hz, H- 13), 7.3-8.2 (10H, m, aryl).

^{13}C-NMR (13): 176.2 (C-6), 35.0 (C-7),42.7(C-8), 83.4 and 78.8 (C- 9/11), 37.9 (C-10), 54.2 (C-12), 142.9 (C- 13), 131.6 (C- 14), 200.1 (C- 15), 41.1 (C- 16), 23.5 (C- 17), 30.4 (weak quint., J= 19.5 Hz, C- 18), 21.4 (weak quint., J= 19.5 Hz, C- 19), 13.6 (C-20); 4-phenylbenzoyl: 166.1, 146.6, 140.2, 130.5, 129.2, 128.5, 128.3, 127.5. No resonances of d_o or d_1 labelled carbon atoms C- 18 or C- 19 are found (2a: 31.4 (C- 18), 22.4 (C-19)).

MS (DI, 70 eV): 192 (6%), 193 (31%, $M^{+\cdot}$ - PBOH- C_4 H_5 D_3), 198 (100%, PBOH), 248 (≤0.2%), 252 (35%, $M^{+\cdot}$- PBOH), 446 (≤ 0.3%), 450 (18%, $M^{+\cdot}$). Utilizing crude 1b in the reaction described above, some 2c is formed as a by-product and easily separated by chromatography (2c: TLC, CH_2Cl_2/EtOAc(10:1), R_f 0.16; m.p. 139-140°C; ^{1}H--NMR: 2.27 (3H,CH_3), ^{13}C-NMR: 27.7(CH_3)).

(dl)-18,18,19,19 ($^2H_4$)- $PGF_{2\alpha}$, 8.

Following known methods (25), the tetradeuterated rac. prostaglandin $F_{2\alpha}$ (8) and its C-15 epimer were prepared. The pure methyl esters of (dl)- 8 (m.p. 66.5°C) and (dl)-15-epi-$PGF_{2\alpha}$ (oil) were analyzed by GC/ MS in the SIM mode (70 eV). The significant fragments (m/z) of the tris-trimethylsilyl ether derivatives proved that unlabelled 8 and 15- epi- $PGF_{2\alpha}$ was undetectable (d_o: 494 (< 0.1%), d_4: 498 ($M^{+\cdot}$- 90); d_o: 404 (< 0.1%), d_4: 408 ($M^{+\cdot}$- 2x 90). In accordance with these findings no ^{13}C-NMR signals of the labelled carbon atoms C-18 and C-19 were recorded (d_o-$PGF_{2\alpha}$ methyl ester: 31.8 and 22.6 respectively (26-28).

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22. Broadening of ^{1}H-NMR resonances in deuterium labelled intermediates has been reported which should be caused by assumed paramagnetic impurities (loc. cit. (7)). In contrast, the broad signals of 1b (H- 4, H- 7) are due to the usual H- D coupling to adjacent CD_2 groups.

23. The assignment is based on the comparition with methyl hexanoate (loc. cit. (24)) and dimethyl 2- oxopropylphosphonate 7: ^{13} C-NMR, 42.4 (dupl., J= 128 Hz, C-1), 200.1 (dupl., J= 5.7 Hz, C- 2), 31.4 (C-3), 53.2 (dupl., J=6.8Hz, OCH_3).

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