SYNTHESIS OF [11β-^{3}H]PROSTACYCLIN

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SUMMARY

The synthesis of [11β-^{3}H]prostacyclin and [11β-^{3}H]6-oxo-prostaglandin $F_{1\alpha}$ has been described. The strategy involved borotritide reduction of prostaglandin D_2, followed by cyclic iodoether formation. Adenylate cyclase activation in a neuronal somatic cell hybrid and GC-MS were used for the analysis of the target compounds. The ready acid catalysed ketal formation of [11β-^{3}H]6-oxo-prostaglandin $F_{1\alpha}$ is noted.

Key words: GC-MS, tritiated prostacyclin, adenylate cyclase, neuronal hybrid cell line.

INTRODUCTION

Prostacyclin (1) is the most potent natural inhibitor of platelet aggregation (1), and its instability (2) makes its quantitation a challenging analytical problem. A programme was initiated for studying the in vivo metabolism of both prostacyclin (1) and its stable hydrolysis product 6-oxo-prostaglandin $F_{1\alpha}$ (3), and to develop quantitative GC-MS assays for their metabolites. A tritium label was required therefore at an accessible, non-metabolic site of the prostacyclin (1) molecule. Prostaglandins readily undergo side-chain oxidation (3) during in

0362-4803/81/030361-10$01.00
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Received September 19, 1979
Revised October 22, 1979

vivo metabolism, thus we sought to introduce the label into the cyclopentane ring.

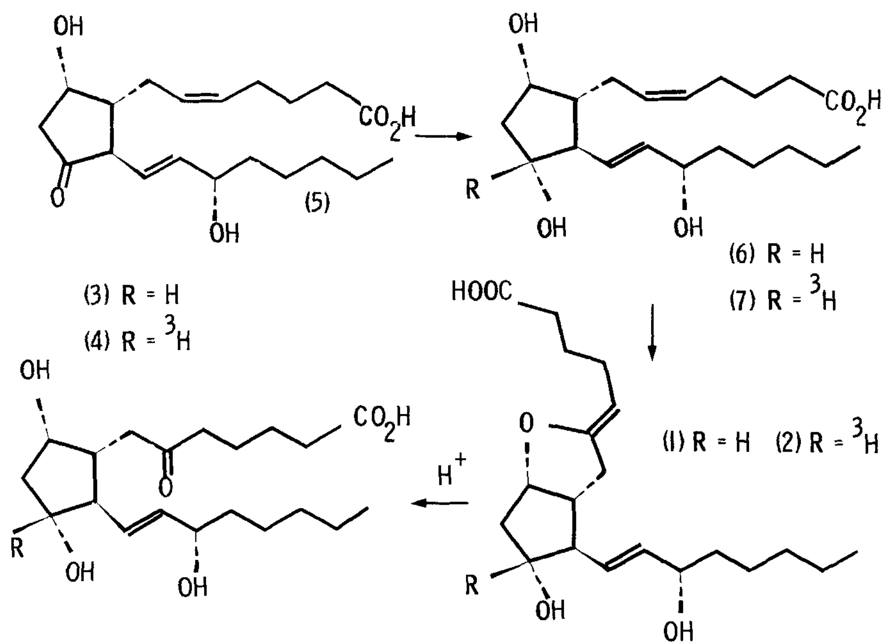

Fig.1.

There are two attractive positions for tritium labelling, namely 9β and 11β, and of these the 11β position was the site of choice for two reasons. Firstly, metabolism of prostacyclin (1) could conceivably proceed by oxidation at C-9, thus causing loss of any tritium label at this position, and secondly, borohydride reduction of prostaglandin D_2 (5) proceeds almost exclusively (Fig.1) to give the natural 11α alcohol (see Experimental), prostaglandin $F_{2\alpha}$ (6). A similar reduction of prostaglandin E_2 (9 ketone), however, produces a 50 : 50 mixture of C-9 epimers which can only be separated by Lipidex chromatography (4). We envisaged utilising the method of Nicolaou (5) for the cyclization of the [11β-^{3}H]prostaglandin $F_{2\alpha}$ (7) to [11β-^{3}H]prostacyclin (2).

We report the synthesis of [11β-^{3}H]prostacyclin (2), the test of its biological activity, and its hydrolysis to [11β-^{3}H]6-oxo-prostaglandin $F_{1\alpha}$ (4), which was analysed by GC-MS.

The Upjohn group (6) (personal communication, unpublished results) have independently completed the synthesis of [11β-^{3}H]prostacyclin. The synthesis of [9β-^{3}H]prostacyclin has appeared recently (7).

EXPERIMENTAL

Materials and Methods

Prostaglandins D_2, $F_{2\alpha}$ and 6-oxo-$F_{1\alpha}$ were kind gifts of Dr J Pike, Upjohn Co.; prostacyclin was a gift of Dr S Moncada, Wellcome Research Laboratories. Sodium [^{3}H]borohydride was purchased from The Radiochemical Centre, Amersham. Thin layer chromatography (t.l.c.) was carried out on pre-coated Merck 0.25 mm silica gel 60 F_{254}, 20 x 20 cm and 5 x 20 cm plates. Radiochromatographic scanning was carried out on a Packard Model 7201. Mass spectra were obtained on a Finnigan 4000 automated gas chromatograph-mass spectrometer (GC-MS) system (3% OV-1 column 2 m x 2 mm (internal diameter), 255°) working at 25 eV.

The NCB-20 mouse neuroblastoma x brain of foetal Chinese hamster hybrid (8) was cultured and prepared for assay of adenylate cyclase activity as described previously (9). Cell homogenates were assayed for adenylate cyclase activity [ATP: pyrophosphate-lyase (cyclizing); EC 4.6.1.1] by a modification (10) of method C of Salomon *et al*. (11), except that incubations of 9 min were performed at 30° and pH 8.5 to minimise the spontaneous hydrolysis of prostacyclin to 6-oxo-prostaglandin $F_{1\alpha}$. Protein was determined by a modification of the method of Lowry *et al*. (12).

[11β-^{3}H]Prostaglandin $F_{2\alpha}$ (7)

Prostaglandin D_2 (5) (5 mg, 15 μmol) in ethanol (1 ml) was treated with

sodium [^{3}H]borohydride (25 mCi, 550 mCi/mmol) at -20° for 15 min then allowed to warm to room temperature over 20 min. Sodium borohydride (5 mg, 0.1 mmol) was added to complete the reaction and excess 2N HCl added dropwise until gas evolution ceased. The reaction mixture was diluted to 50 ml with water, the pH adjusted to 3, and extracted with redistilled ethyl acetate (3 x 50 ml). The organic extracts were evaporated to dryness *in vacuo* at 40° and the residue desiccated under vacuum (30 min). The crude free acid was dissolved in methanol (1 ml) and converted to its methyl ester with ethereal diazomethane. Purification was carried out by chromatography on Lipidex 5000, eluting with heptane : chloroform 80 : 20 v/v (4), to give pure [11β-^{3}H]prostaglandin $F_{2\alpha}$ (7) (3 mCi) as its methyl ester. It has been shown (13) that the methyl esters of 11α- and 11β-H-prostaglandin $F_{2\alpha}$ are separated under these conditions. The major peak corresponded to authentic 11β-H-prostaglandin $F_{2\alpha}$ methyl ester.

[11β-^{3}H]Prostacyclin (2)

Prostaglandin $F_{2\alpha}$ (6) methyl ester (30 mg, 84 µmol) was dissolved in dichloromethane (2 ml) and diluted with [11β-^{3}H]prostaglandin $F_{2\alpha}$ (7) methyl ester (600 µCi). The solution was cooled to -10°, then treated with anhydrous potassium carbonate (28 mg, 0.16 mmol) and stirred while a solution of iodine (28 mg, 0.1 mmol) in dichloromethane (0.25 ml) was added. The reaction mixture was stirred for 24 h at 0°, filtered and evaporated to give the crude [11β-^{3}H]-iodo ether which was purified chromatographically on two 20 x 20 cm, 0.25 mm silica 60 F_{254} plates, developing with methanol : ether (1 : 9). The purified material was taken up in ethanol (1 ml) and a solution of sodium ethoxide in ethanol (0.2 ml, 0.5 g/10 ml) was added. The dehydrohalogenation was completed by heating to 75° for 1 h. The reaction mixture was then cooled to room temperature, saponified by adding water (0.2 ml) and left to stand overnight. The [11β-^{3}H]prostacyclin (2) was obtained as its sodium salt (21 mg, 382 µCi, 7 mCi/mmol) and stored in water : ethanol (1 : 9) at -70°.

[11β-^{3}H]6-oxo-Prostaglandin $F_{1\alpha}$ (4)

The [11β-^{3}H]prostacyclin (2) sodium salt (2 mg, 40 µCi) in water (2 ml) was acidified with 0.2 N HCl to pH 3 using a pH meter. The aqueous solution was immediately extracted with ethyl acetate (2 x 10 ml), dried, filtered and evaporated to give the [11β-^{3}H]6-oxo-prostaglandin $F_{1\alpha}$ (4) (38 µCi). The t.l.c. indicated a single compound, which corresponded to authentic 6-oxo-prostaglandin $F_{1\alpha}$ (3) both by vanillin/H_2SO_4 spray and radiochromatogram scanning.

RESULTS AND DISCUSSION

Prostaglandin D_2 (5) was reduced smoothly with sodium [^{3}H]borohydride, and the [11β-^{3}H]prostaglandin $F_{2\alpha}$ (7) separated as its methyl ester from inorganic and organic by-products by chromatography on Lipidex 5000. Dilution of this product with unlabelled cold prostaglandin $F_{2\alpha}$ was followed by iodoether formation, dehydrohalogenation and saponification using the method of Nicolaou (5) to give [11β-^{3}H]prostacyclin (2) as its sodium salt. In order to test the purity of this product a portion was hydrolysed at pH 3, extracted into ethyl acetate and run on t.l.c.

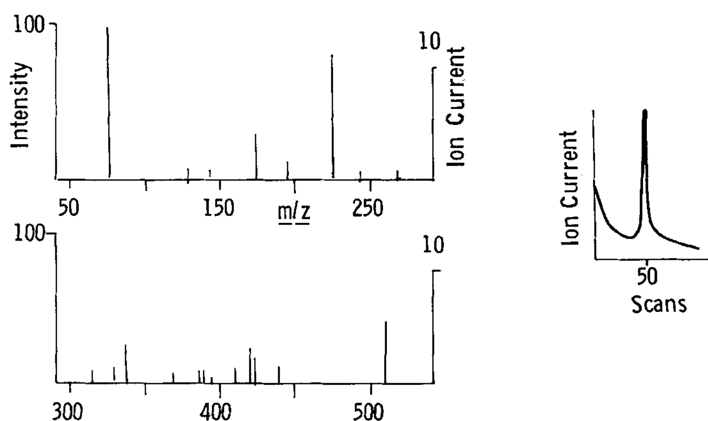

Fig.2. Total ion current and mass spectrum showing the major peaks of the di-TMS ether, methyl ester of [11β-^{3}H]6-methoxy-prostaglandin I_1. The scales of intensity and ion current have been multiplied by a factor of 16 for m/z > 360.

The radiochromatogram indicated that a single compound had been formed. This band was eluted with methanol and derivatised (methoxyamine hydrochloride, diazomethane, N,O-bis(trimethylsilyl)-trifluoroacetamide [BSTFA]) for analysis by GC-MS. The reconstructed total ion current indicated that a single compound had been formed (Fig.2), however the mass spectrum was not consistent with that of the expected derivative of 6-oxo-prostaglandin $F_{1\alpha}$ (10). A close inspection of the data allowed assignment of the structure as the di-trimethylsilyl (di-TMS) ether, methyl ester of 6-methoxy-prostaglandin I_1 (9). The mass spectrum (Fig.2) showed an ion at m/z 510 which is consistent with the loss of methanol from a molecular ion at m/z 542. The ions at the upper mass range could be readily assigned: m/z 439 M-(MeOH + C_5H_{11}), m/z 423 M-(MeOH + CH_2CH_2COOMe), m/z 420 M-(MeOH + TMSOH), m/z 389 M-(MeOH + MeO + TMSOH), m/z 330 M-(MeOH + TMSOH + TMSOH).

A plausible mechanism for the formation of the ketal (8) involves an acid catalysed reaction of methanol at the ketone function of 6-oxo-prostaglandin $F_{1\alpha}$ (4). Subsequent intramolecular cyclization from the 9α-hydroxyl group, with displacement of water, would provide the observed product. Treatment of authentic prostacyclin (1) in the same way, resulted in the formation of an identical product. This ketal (8) was originally observed by Sih (14) as its methyl ester in the reaction of diazomethane with 6-oxo-prostaglandin $F_{1\alpha}$ (3).

The hydrolysis product for t.l.c. was derivatised (methoxyamine hydrochloride, diazomethane, BSTFA), rather than subjecting it to chromatography. GC-MS analysis indicated that a different compound with a longer retention time (9.2 min) had been formed (Fig.3). The mass spectrum was consistent with that of the methoxime, tri-TMS ether, methyl ester of 6-oxo-prostaglandin $F_{1\alpha}$ (10).

The molecular ion appeared at m/z 629, and the other major ions in the

spectrum could be assigned as fragments from the molecular ion: m/z 614 M-Me, m/z 598 M-OMe, m/z 558 M-C_5H_{11}, m/z 508 M-(OMe + TMSOH), m/z468 M-(TMSOH + C_5H_{11}), m/z 449 M-(TMSOH + TMSOH), m/z 418 M-(TMSOH + TMSOH + OMe), m/z 378 M-(C_5H_{11} + TMSOH + TMSOH). Authentic prostacyclin (1) was treated in the same way and an identical product was formed.

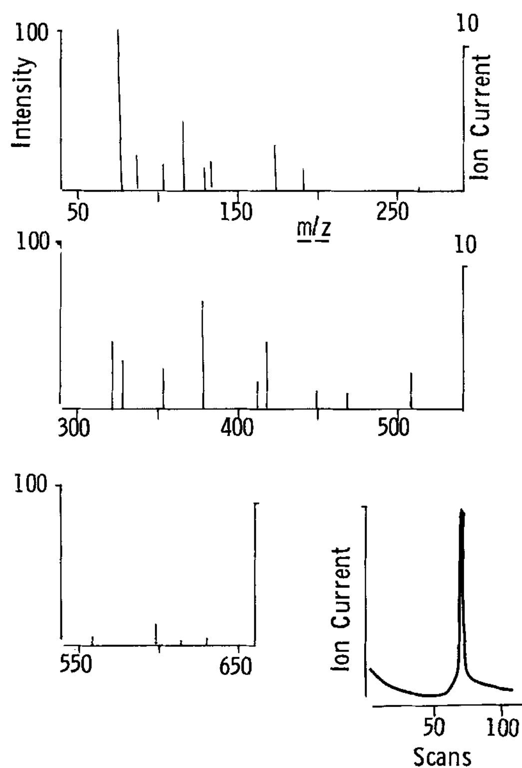

Fig.3. Total ion current and a mass spectrum showing the major peaks of the tri-TMS ether, methoxime, methyl ester of [11β-³H]6-oxo-prostaglandin $F_{1\alpha}$. The scales of intensity and ion current have been multiplied by a factor of 8 for m/z > 320.

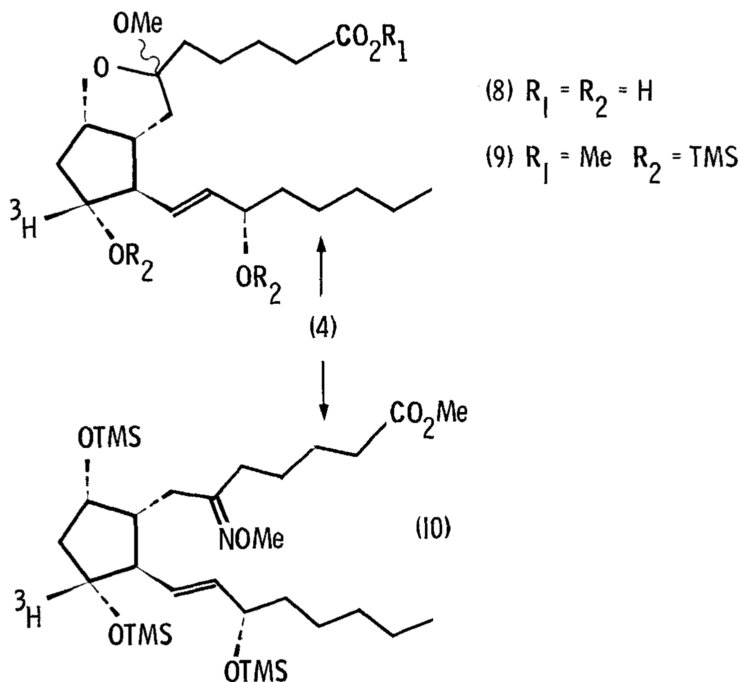

Fig.4.

Although acid catalysed ketal formation is a very facile process, the GC-MS traces indicated that hydrolysis of [11β-^{3}H]prostacyclin (2) produced essentially pure [11β-^{3}H]6-oxo-prostaglandin $F_{1\alpha}$ (4). Having established the chemical purity of labelled prostacyclin, the biological activity was assessed. The [11β-^{3}H]prostacyclin (2) was compared with authentic prostacyclin (1) and its hydrolysis product 6-oxo-prostaglandin $F_{1\alpha}$ (4) for activation of adenylate cyclase in a neuronal somatic cell hybrid. The shapes and positions of the curves (Fig.5) for synthetic and authentic prostacyclins were almost identical. An Eadie-Hofstee plot gave a $K_{act.}$ = 28 nM for authentic prostacyclin and $K_{act.}$ = 36 nM for [11β-^{3}H]prostacyclin ($K_{act.}$ = concentration of prostaglandin, producing half-maximum activation).

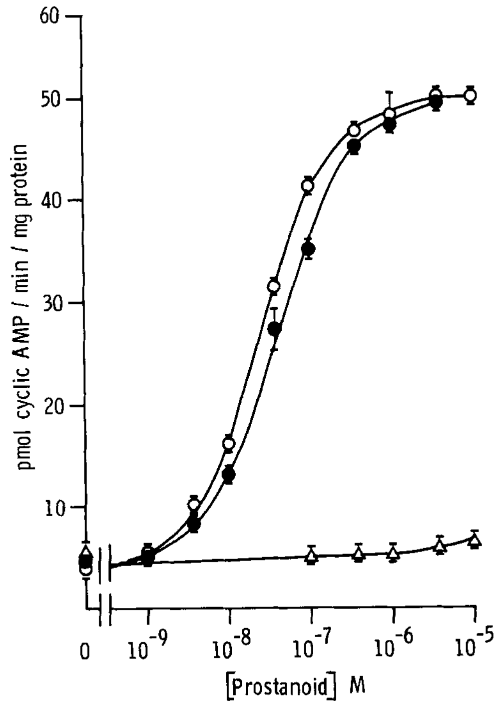

Fig.5. Prostacyclin-dependent activation of adenylate cyclase in a homogenate of NCB-20 hybrid cells. Results show the means (± SEM) of triplicate determinations of adenylate cyclase activity in the presence of increasing concentrations of authentic prostacyclin (○), [11β-^{3}H]prostacyclin (●) and 6-oxo-prostaglandin $F_{1\alpha}$ (Δ).

The synthesis of [^{3}H]prostacyclin and [^{3}H]6-oxo-prostaglandin $F_{1\alpha}$, with the label in the 11β position provides chemically pure compounds of known biological activity. We look forward to utilising these materials in metabolic studies.

ACKNOWLEDGEMENTS

We thank Mr R Clare for operating the GC-MS system; Professor C T Dollery for useful discussions; Dr M Nirenberg (National Institutes of Health, USA) for

the NCB-20 hybrid cell line.

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