SYNTHESIS OF DEUTERATED EICOSANOIDS

Claus O.Meese

Dr.Margarete Fischer-Bosch-Institut für Klinische
Pharmakologie, Auerbachstraße 112, D-7000 Stuttgart 50 (FRG)

SUMMARY

Deuterated lipoxygenase and cyclooxygenase derivatives of eicosatetraenoic acid (arachidonic acid) and their metabolites are indispensable tools for organic trace analysis, biomedical studies, and mechanistic investigations on the corresponding endogenous (unlabelled) eicosanoids. Methods for the preparation of deuterated eicosanoids are reviewed.

Key words: Eicosatetraenoic acid metabolites, prostaglandins, thromboxanes, deuteration, mass spectrometry

INTRODUCTION

A basic requirement for the trace analysis of eicosanoids (1,2) in biological material using the mass spectrometric stable isotope dilution method (3-6) is the availability of isotopically labelled internal analytical standards (6,7). As a variety of deuterated precursors or reagents are readily available commercially, most work done in the past was based on the preparation of deuterated analogues. For their synthesis and use some general considerations have to be taken into account:

1. The labelled compounds should preferably contain at least 3-4 deuterium atoms.
2. The label should be placed in chemically stable positions to avoid H/D exchange during storage, sample work-up or chromatography*.
3. The label should be retained in the main mass fragments after ionization of an appropriate derivative in the ion source of the mass spectrometer.
4. The amount of unlabelled material ($^{2}H_{o}$) in the internal standard should be as low as possible to obtain a sensitive, low-blank assay.
5. For an economic synthesis the deuterium should be introduced into the molecule at a late stage of the synthetic route.

0362-4803/86/030295–18$09.00
© 1986 by John Wiley & Sons, Ltd.

Received August 26, 1985
Revised October 9, 1985

6. If the deuterated compound is to be used in biological studies (biosynthesis, metabolism, bioavailability, etc.), the enzymatic stability has to be considered (8,9).

Deuterated eicosanoids may also be useful in mechanistic and spectroscopic investigations (elucidation of reaction mechanisms (10), fragmentation mechanisms in mass spectrometry (11,12), and signal assignment in ^{1}H, ^{13}C-NMR spectroscopy (13)).

This contribution describes the general routes to biologically relevant eicosanoids which are deuterated at stable positions of the carbon skeleton and which are suitable for use as analytical primary standards. Despite some analytical limitations, the preparation of secondary standards (deuterated derivatives of the unlabelled parent compounds) will also be outlined briefly.

LABELLED PRECURSORS AND INTERMEDIATES

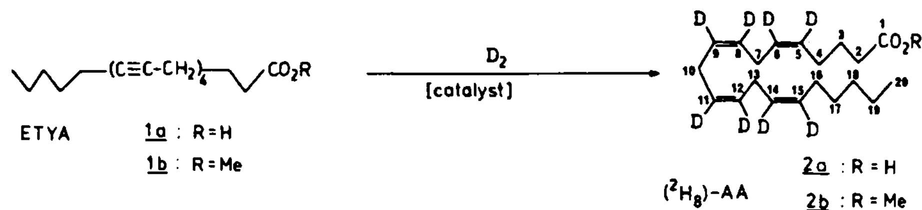
The majority of the physiologically active lipoxygenase or cyclooxygenase derivatives is derived from the common precursor arachidonic acid (AA, eicosa-5,8,11,14-tetraenoic acid). Although the synthesis of a selectively monodeuterated AA has also been

---

*Abbreviations used in this article: ^{2}H=D, arachidonic acid = AA, prostaglandin = PG, leukotriene = LT, hydroxyeicosatetraenoic acid = HETE, thromboxane = TX

Nomenclature and numbering: prostaglandins (14), prostacyclins (15) thromboxanes (16), leukotrienes (17)

described (18), most preparations of deuterated AA start from eicosa-5,8,11,14-tetraynoic acid 1a (ETYA) (19,20) or its methyl ester 1b. (Scheme 1).



Scheme 1

Quinoline poisoned Lindlar's catalyst (21-23) is widely used for the semi-saturation of the triple bonds of 1 (19,20,24,25) although it has been shown by careful chromato-

graphy (26,27) that the chemical yield and isotopic purity of 2 have often been largely overestimated (28). P-2 nickel (29,30) is also effective for the cis-deuteration of 1b (31,32) while little or no deuterium uptake was observed with other catalysts ($(Ph_3P)_3RhCl/CF_3CH_2OH$ (33), $(Rh(NBD)Me_2PPh)^+$ PF_6^- (34), Co_2B (35)) (28). As determined by capillary gas chromatography-mass spectrometry, Lindlar semi-deuteration provides 5,6,8,9,11, 12,14,15-($^2H_8$)-arachidonic acid in 45-75 % yield with an isotopic distribution in the range of 3% $^2H_6/^2H_{10}$,10-20 % $^2H_7/^2H_9$, 59-86% $^2H_8$ ($d_o \leqslant 0.5\%$) (25,28,36).

The total synthetic approach to labelled eicosanoids requires the introduction of labelled side chains. In an early report (37,38) an efficient synthesis of the phosphonium salt 4 from commercial deuterated cyclopentanone 3 was described (Scheme 2). Compound 4 may also be conveniently prepared in five steps starting from 5-acetoxy-1-methoxy-2-pentyne 5 (39).

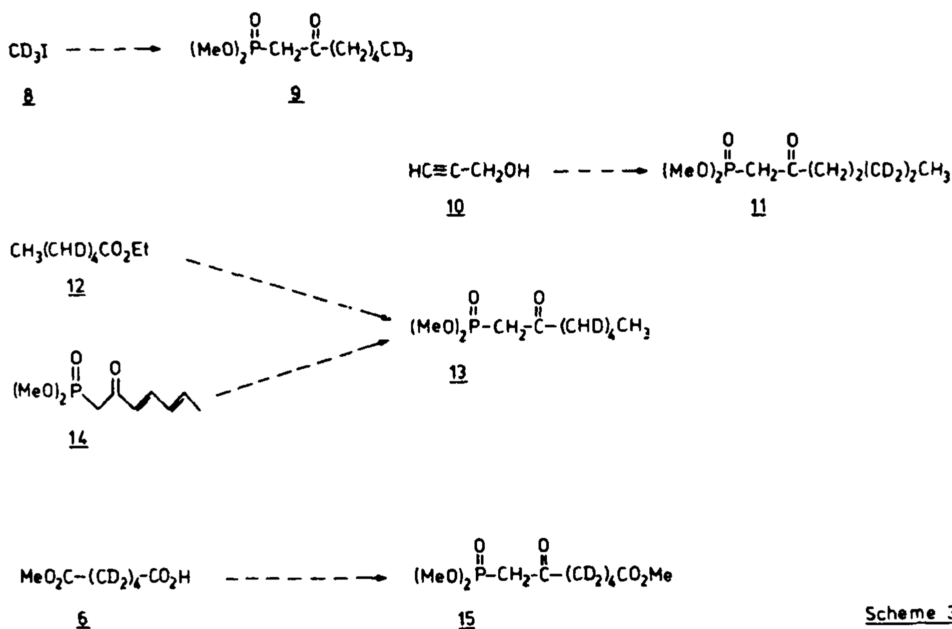
Scheme 2

The highly labelled phosphonium salt 7 has recently been pepared(40) from 6(41) via the Hunsdiecker reaction.

The Wittig precursor 4 has been widely used for the introduction of the upper side chain (see below) to yield prostanoids labelled at the positions 3,3,4,4 of the C-20 carbon skeleton (see below).

However, as a number of prostanoic acid metabolites possess a shortened upper side chain deuterium introduction into the lower side chain of the carbon skeleton via the Wittig-Horner reaction of appropriate phosphonates seemed to be more favourable in these cases. Thus, the requisite phosphonates 9 (42), 11 (43), and 13 (40,44) (Scheme 3)

have been obtained in satisfactory yields from readily available starting materials (12 (44) and 14 (40,45) may be conveniently prepared from sorbic acid). A key step in the synthesis of 11, 13, and 15 is the saturation of double or triple bonds by means of homogeneous catalytic deuteration. Without exception, Wilkinson's catalyst ($(Ph_3P)_3RhCl$) (46) has been used for this step. The octadeuterated phosphonate 15 has been used in the total synthesis of labelled ω-carboxy-metabolites of prostaglandins (40) and thromboxanes (41).



Scheme 3

BIOCHEMICAL TOTAL SYNTHESIS

In the past twenty years, a variety of enzymes which are responsible for each of the individual steps of the arachidonate cascade have been localized in the animal and plant kingdom(47,48).

However, because of the difficulties encountered with the purification or isolation of enzymes, biosynthetically less specific cell homogenates or subcellular fractions were employed for the generation of deuterated ecosanoids. Thus, the widely used precursor 2a has been converted to a number of physiologically relevant labelled eicosanoids (Table 1).

Table 1: Biosynthesis of eicosanoids from 2a

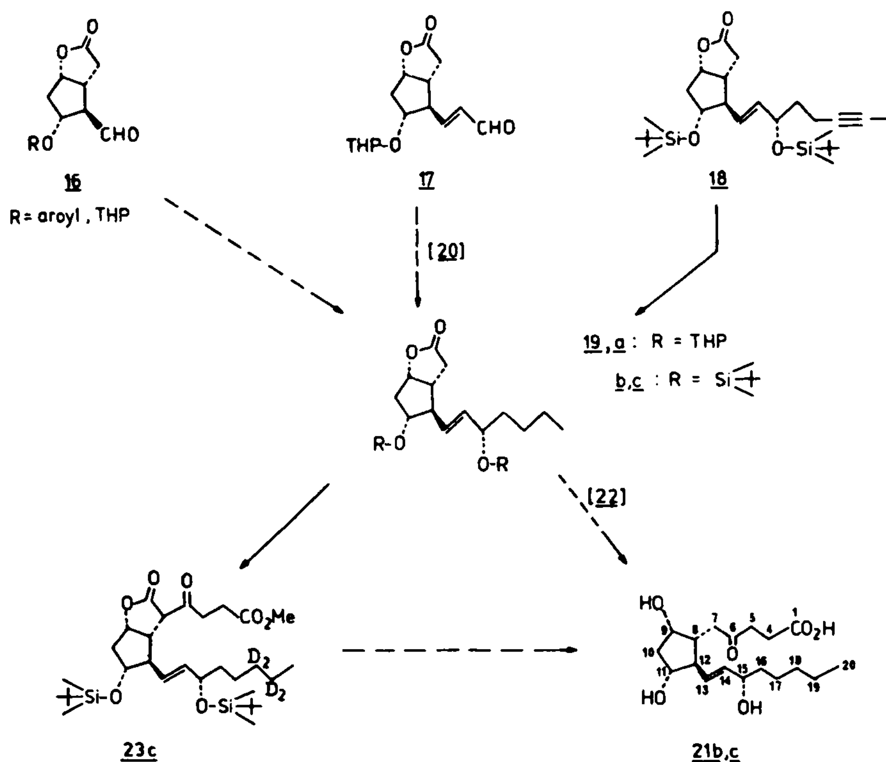
source of enzymes	product(s)	References
sheep seminal vesicles	$^{2}H_{7}$-PGE_{2}	49 - 51
homogenates of rat spleen, sheep vesicular gland	$^{2}H_{6}$-PGD_{2},$^{2}H_{8}$-12-HETE, $^{2}H_{8}$-15-HETE	52,53
ram seminal vesicles	$^{2}H_{6}$-6-oxo-PGF_{1a}	54
rat stomach homogenates	$^{2}H_{7}$-6-oxo-PGF_{1a}	55,56
platelets	$^{2}H_{8}$-12-HETE,d_{8}-TXB_{2}	52,57,58
homogenates of bovine lung	$^{2}H_{8}$-TXB_{2}	53
rat peritoneal mast cells	$^{2}H_{6}$-PGD_{2}	59
polymorphonuclear leukocytes	$^{2}H_{8}$-5-HETE,$^{2}H_{8}$-LTB_{4}	53
soybean lipoxygenase	$^{2}H_{8}$-15-HETE	53

The major advantage of this total biosynthetic approach is the extremely short (mainly one step) access to a variety of labelled eicosanoids. At the same time, some severe disadvantages have to be taken into account. Yields are generally moderate to poor, extensive chromatography is essential to remove excess endogenous impurities, only small batches (in the µg range) may be handled, and loss of deuterium is often observed. When unlabelled arachidonic acid is liberated from the endogenous lipid pool, the products isolated after incubation of 2a are often severely "contaminated" with the corresponding unlabelled metabolite. These preparations are therefore of limited value as internal standards in a mass spectrometric trace assay. However, recent advances in high performance high pressure liquid chromatography have demonstrated that argentation of a suitable stationary phase permits the separation of some unlabelled and deuterated eicosanoids when the label is linked to an olefinic bond (53).

CHEMICAL TOTAL SYNTHESIS

The rapidly expanding synthetic prostaglandin methodology (60) has largely facilitated and accelerated the progress in the chemical synthesis of deuterated prostanoids. The

extremely versatile Corey's aldehyde 16 (61) serves as common starting material for the preparation of the intermediates 17 (61,62), 18 (63), and 19 (37,38,62-64) (Scheme 4).



Scheme 4

a : 3,3,4,4-($^2H_4$); b : 19,19,20,20-($^2H_4$) ; c : 18,18,19,19-($^2H_4$) ; d : 16,17,18,19-($^2H_4$)

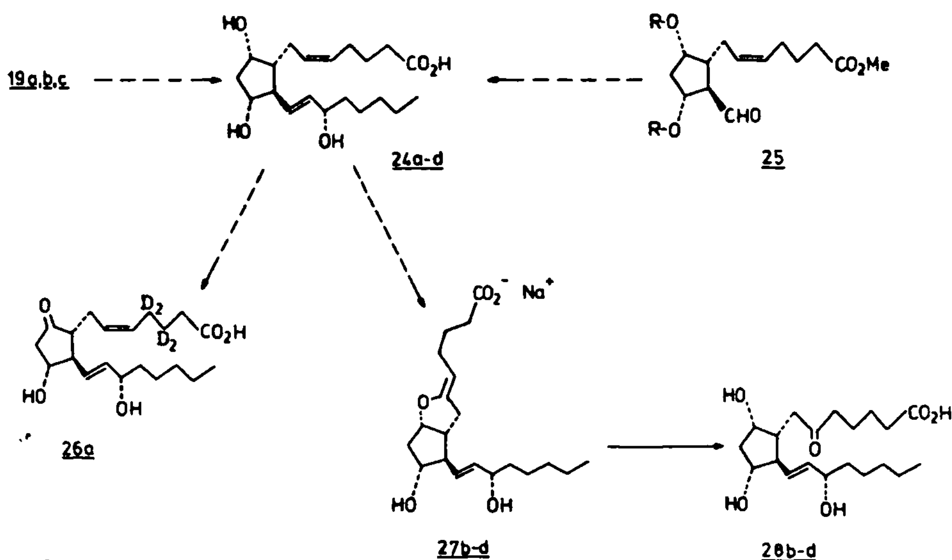
20 : $Br-(CH_2)_3CO_2CD_2H$; 22 : $(Me_2N)_2P(O)OCH_2CH=CH_2$

Deuterium incorporation was achieved via homogeneous catalytic deuteration of 18 (using Wilkinson's catalyst) (63) or coupling of 16 (64) or 17 (62) with the labelled intermediates 11 or 20, to give 19c and 19b, respectively.

Utilization of the intermediate lactones 19 allowed the two step syntheses of labelled 2,3-dinor-6-oxo-PGF$_{1a}$ 21b (62) and 21c (64) using the spiro-lactonization reagent 22 (62) or via deprotection and decarboxylation of the acyl-lactone 23 (64,65).

As illustrated in Scheme 5, construction of the whole prostaglandin framework was

accomplished by conventional Wittig reaction of 19 (generated from 4) either with an unlabelled or a deuterated ylide to give the F-prostaglandins 24. Alternatively, the labelled ω side-chain blocks of 11 and 13 could be coupled to 25 to give the deuterated prostaglandin F_{2a} isotopomers 24c (43) and 24d (44), respectively. Following prostaglandin interconversion strategies, several C-20 primary prostaglandins are accessible from 24. Thus, the syntheses of labelled PGE_2 (26a) (37,38), prostacyclin I_2 (27), and 6-oxo-PGF_{1a} (28) have been achieved (13,44,62,66).

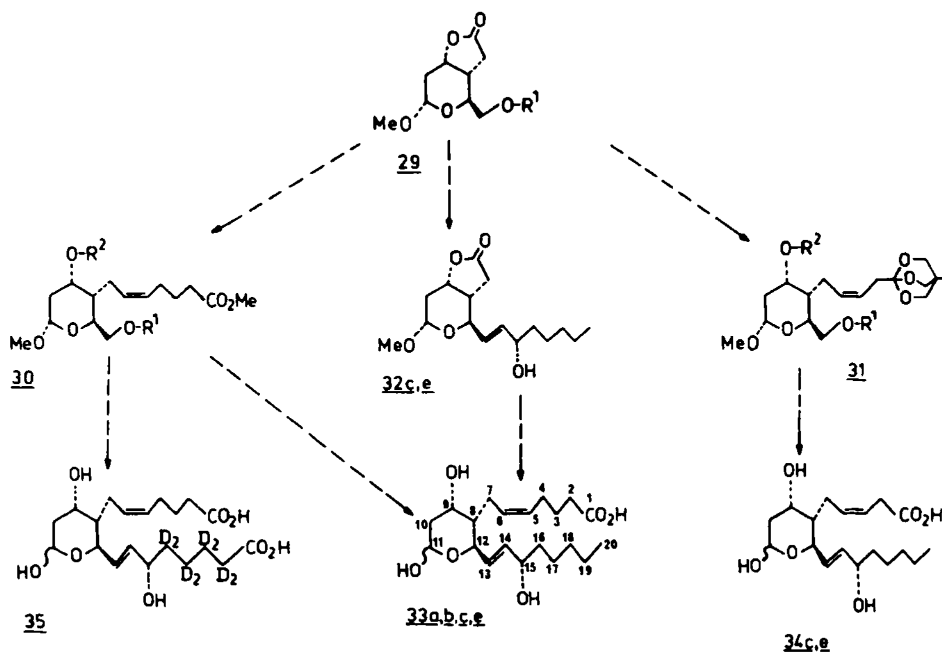


Scheme 5

a : 3,3,4,4-($^2H_4$); b : 19,19,20,20-($^2H_4$) ; c : 18,18,19,19-($^2H_4$) ; d : 16,17,18,19-($^2H_4$)

The chemical syntheses employed in the thromboxane series are depicted in Scheme 6. The synthetically versatile building block 29 (67-71) has served as starting material for the initial α-substitution (30,31) (39,41,69,71,72) or ω-substitutuion (32) (67,73,74). Completion of the frameworks of thromboxane B_2 (33) (39,70,73,74), the 2,3-dinor metabolite 34 (71-74), and the ω-carboxy metabolite 35 (41) were effected by use of the appropriate deuterated phosphonates or phosphonium salts (see Schemes 2,3).

It should be noted that ring-enlargement of partially protected 24b (75) according to previous methods elaborated for the synthesis of unlabelled thromboxane B_2 (76) yields 33b.



Scheme 6

a : 3,3,4,4-($^2H_4$); b : 19,19,20,20-($^2H_4$)

c : 18,18,19,19-($^2H_4$); e : 20,20,20-($^2H_3$)

R^1 = H, Si≦+, $SiPh_2$t-Bu

R^2 = Ac, Bz

Although chemical syntheses of leukotrienes and other lipoxygenase products have been extensively elaborated (77,78), little is known about the deuterated analogues. A tedious multi-step total synthesis led to (±)-5,6,8,9-$^2H_4$-12-HETE (79).

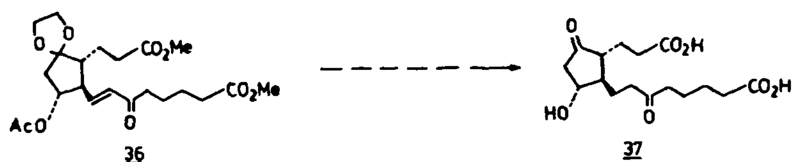
A one-pot preparation of different octadeuterated, racemic HETEs by a "biomimetic" copper catalyzed autoxidation of $^2H_8$-AA has been published (80). Although the yields of the individual products are low and extensive purification is essential, this procedure represents the most rapid access to labelled HETEs.

The availability of dehydro-leukotriene A_4 analogues (81-84) however, should enable the preparation of a variety of at least dideuterated leukotrienes. This approach has been utilized in the synthesis of 14,15-$^3H_2$-LTE_4 via partial tritiation (Lindlar) of 14,15-dehydro-LTA_4 methyl ester (84).

SEMISYNTHESIS

The semisynthetic approach is based either on chemical or biochemical modification of

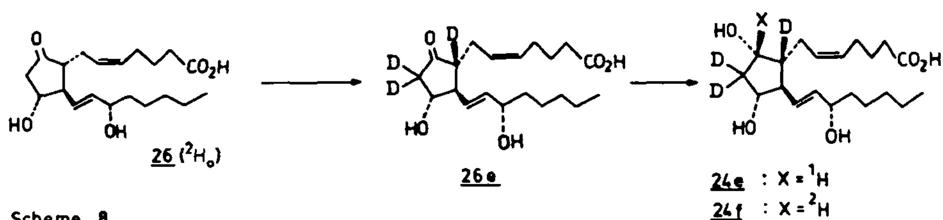
deuterated (and occasionally partially protected) eicosanoids or on deuterium introduction (by H/D exchange or other chemical methods) into unlabelled eicosanoid. A combination of double-bond deuteration and base catalyzed H/D exchange using the prostaglandin intermediate 36 (85) furnished after protective group removal pure crystalline 37 (($\pm$)-PGE-M, the major urinary metabolite of PGE_1 and PGE_2). Although extensive scrambling had occurred, 37 exhibits an isotopic maximum of $^2H_7$ (86) (Scheme 7).



Scheme 7

A related, relatively short half-life metabolite of PGE_2 in the circulation (13,14-dihydro-15-oxo-PGE_2) could be isolated as a low-blank ($^2H_0$:$^2H_4$=0.2-0.35%) deuterated analogue (3,3,4,4-$^2H_4$) in 8-16% yield after incubation of 26a with a subcellular fraction of either swine kidney or guinea pig liver (87). In a similar manner, the corresponding F-metabolite was prepared from 2a (38).

Another semisynthetic route to various deuterated prostanoids utilizes the known base--catalyzed enolization of the 9-oxo group of E-prostaglandins (Scheme 8).



Scheme 8

Subsequent borohydride (^1H,^2H) reduction of 26e provides 24e,f in preparative scale after chromatographic removal of the 9β epimers (13,88-91). The ring labelled F-prostaglandins 24e,f have been converted into the corresponding labelled prostacyclin 27 and 6-oxo-PGF_{1a} 28, as outlined in Scheme 4 (13,89,90). The methyl ester of 24f was

chosen for a careful determination of the isotopic distribution and site of labelling. Using various methods (7), preferably soft-ionization techniques like field desorption mass spectrometry (FD-MS) (92), an isotopic composition of 2% $^2H_1$, 9 % $^2H_2$, 41 % $^2H_3$, 46 % $^2H_4$, 2 % $^2H_5$ ($\leqslant$ 0.2% $^2H_0$ referred to $^2H_4$) was established (13). The position responsible for the incomplete deuteration was localized by proton-decoupled ^{13}C-NMR spectroscopy. The rather incomplete (30-50%) labelling at C-8 of 24f, identified by this technique, is indicative of a dominating enolization between C-9 and C-10 of 26 (13).

Other carbonyl or double bond reduction reactions have also been shown to be useful for deuterium introduction to prostanoids, e.g. reduction of 15-oxo-prostanoids with sodium borodeuteride (11,93), which, however, yields a mixture of epimeric alcohols, or chemospecific homogeneous catalytic deuteration of the 5,6-double bond of A,B,E,F-prostaglandins in which the 13,14-double bond has been shielded by silylation of the allylic 15-hydroxy group (94,95).

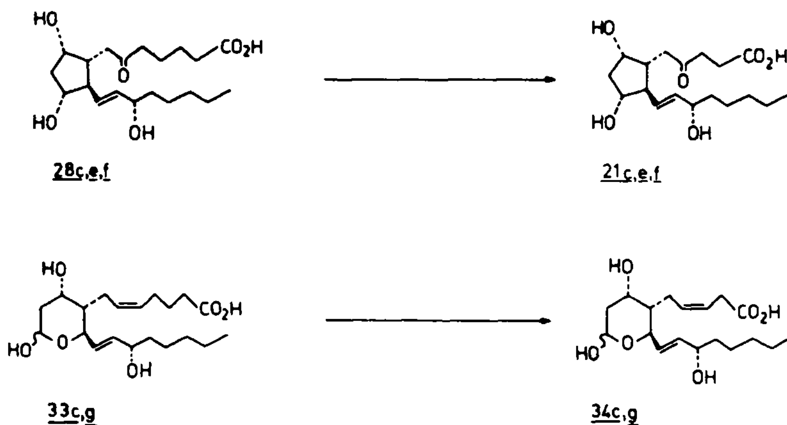
The known interconversion reactions of prostanoids also allow the preparation of labelled PGE_2 (Scheme 5) and PGA_2 (96) from deuterated PGF_{2a} and PGE_2, respectively.

Because ß-oxidation is a dominating pathway in endogenous eicosanoid metabolism, isotopically labelled 2,3-dinor metabolites were required as diagnostic index standards. Before completion of the chemical total synthesis (Scheme 4,6), the desired labelled major human metabolites of PGI_2 and TXA_2, 21 and 34, were prepared by microbial (Mycobacterium Rhodochrous) (97, 98) ß-oxidation of the deuterated C-20 precursors 28 and 33 (73,74,89,90) (Scheme 9).

As the label is placed in the lower side chain (28c, 33c,g) or in the cyclopentane ring (28e,f), complete deuterium retention is achieved.

MISCELLANEOUS

Although beyond the scope of this article, some other analytically useful preparations of labelled eicosanoids should be briefly mentioned. Oxygen-18 introduction into the carboxylic group of various eicosanoids has been achieved by hydrolytic ($Li^{18}OH$) or enzymatic (porcine liver esterase, $H_2{}^{18}O$) cleavage of their methyl esters (Scheme 10). Re-esterification and repetition of the ^{18}O incorporation during several cycles led to $^{18}O_2$-carboxylic acids of satisfactory isotopic purity (99-103).



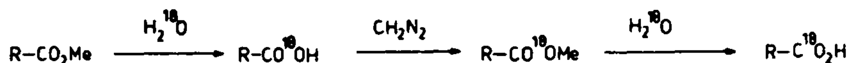
Scheme 9

21, 28 : c, 18,18,19,19-($^2H_4$); e, 8,10,10-($^2H_3$); f, 8,9,10,10-($^2H_4$)

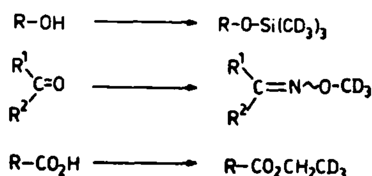
33, 34 : c, 18,18,19,19-($^2H_4$); g, 20,20,20-($^2H_3$)

The isotopic stability of these isotopomers in aqueous media, however, is limited (99).

The hydroxy-, carboxy-, or oxo groups of appropriate eicosanoids may be derivatized with deuterated reagents to provide labelled "secondary standards" which are added to the analytical sample after derivatization of the analyte with the corresponding unlabelled reagent. F-prostaglandins have been converted into their tris (trideuteromethyl) silyl ethers (104,105) while O-(trideuteromethyl) hydroxylamine treatment converts E-prostaglandins (105,106,107), D-prostaglandins (40), or 15-oxo metabolites (108) into the deuterated oximes (Scheme 11).



Scheme 10



Scheme 11

Similarly, treatment of eicosanoids (with the exception of sulfidopeptide leukotrienes (109)) with deuterated diazomethane (110,111) provides dideutero methyl esters (113).

To avoid scrambling, D/H-back exchange, and a high blank, a synthesis of the new 2,2,2-trideutero-diazoethane has been developed (114) which permits the quantitative generation of low-blank trideuteroethyl esters of e.g. TXB_2,12-HETE,PGD_2, and two PGF,E metabolites (Scheme 11,R: eicosanoid). These easily prepared secondary standards proved useful in the mass spectrometric quantification in biological samples (115).

CONCLUSION

The enzymatically directed biochemical total synthesis of deuterated eicosanoids undoubtably belongs to the most rapid synthetic methods. However, the amounts available (usually in the μg range) are generally limited. Many in vitro or in vivo conversions of deuterated starting materials exhibit severe dilution with unlabelled endogenously produced metabolites (54). Finally, the presence of closely related impurities as well as interconversion products (administered E- or D-prostanoids give often rise to the formation of F-prostanoids (87)) necessitates very careful purification. In contrast, chemical total synthesis of deuterated eicosanoids include many laborious steps. Despite this obstacle, the availability of several key intermediates and, specifically, deuterated side chain blocks creates great flexibility in the planned synthesis of the desired final products. The economic and highly efficient (yield $\geqslant$ 90%, $^2H_4 \geqslant$ 92%) homogeneous deuteration using Wilkinson's catalyst ($(Ph_3P)_3RhCl$) of the triple bond of acetylenic precursors provides low-blank eicosanoids of high isotopic purity. It is expected that the methodology presented above for the synthesis of deuterated eicosanoids will be extended to the rapidly growing list of recently detected eicosanoids, other closely related fatty acids (116), or biologically active analogues of pharmaceutical interest (117,118). Irrespective of the mass spectrometric method applied, deuterated eicosanoids will maintain their versatile value in chemical or biological investigations.

ACKNOWLEDGEMENTS

This work was supported by the Robert-Bosch-Stiftung (Stuttgart) and a grant (Me 792/1-1) of the Deutsche Forschungsgemeinschaft (Bonn), which is gratefully acknowledged. Thanks go also to Mrs.Grözinger and Dr.Dunmur for typing and revising the manuscript, respectively.

REFERENCES

1. Green K., Hamberg M., Samuelsson B. and Frölich J.C.- Advances in Prostaglandin and Thromboxane Research (Ed.J.C.Frölich), Raven Press New York, Vol. 5, p.39 (1978)
2. Granström E.- The Prostaglandin System (Ed.F.Berti and G.P.Velo), Plenum Press, New York, Vol. 36, p.67 (1981)
3. Sweeley C.C., Elliott W.H., Fries I. and Ryhage R.- Anal. Chem. 38: 1549 (1966)
4. Watson J.T.- Introduction to Mass Spectrometry, Raven Press, New York, 1976
5. Self R.- Biomed. Mass Spectrom. 6: 315 (1979)
6. Haskins N.J.- Biomed. Mass Spectrom. 9: 269 (1982)
7. Millard B.J.- Quantitative Mass Spectrometry, Heyden and Sons Ltd., p.60 (1979)
8. Rittenberg D., Schoenheimer R. and Evans E.A.,Jr.- J.Biol.Chem. 120: 503 (1937)
9. Axen U., Baczynski L., Duchamp D.J., Kirton K.T. and Zieserl J.F.-Adv. Biosci. 9: 109 (1973)
10. Bigeleisen J.- Science 110: 14 (1949)
11. Horvath G. and Ambrus G.- Biomed. Mass Spectrom. 5: 544 (1978)
12. Fischer C. and Meese C.O.- 5. Intern. Conf.Prostaglandins, Florence (Italy), May 18-21 (1982), P.No.38
13. Meese C.O.- J.Lab.Comp. Radiopharm. 20: 817 (1983)
14. Nelson N.A. - J.Med.Chem. 17: 911 (1974)
15. Johnson R.A., Morton D.R. and Nelson N.A.-Prostaglandins 15: 737 (1978)
16. Samuelsson B., Hamberg M., Roberts L.J., II, Oates J.A. and Nelson N.A.- Prostaglandins 16: 857 (1978)
17. Samuelsson B., Borgeat P., Hammarström S. and Murphy R.C.-Prostaglandins 17: 785 (1979)
18. Corey E.J. and Lansbury P.T.,Jr.- J.Am. Chem.Soc. 105: 4093 (1983)
19. Osbond J.M., Philpott P.G. and Wickens J.C.- J.Chem.Soc.2779 (1961)

20. Fryer R.I., Gilman N.W. and Holland B.C.-J.Org.Chem. 40: 348 (1975)

21. Lindlar H.- Helv.Chim.Acta 35: 446 (1952)

22. Marvell E.N. and Li T.- Synthesis 457 (1973)

23. Adlof R.O. and Emken E.A.- J.Lab.Comp. Radiopharm. 21: 75 (1984)

24. Hamberg M., Niehaus W.G.,Jr. and Samuelsson,B.- Anal.Biochem. 22: 145(1968)

25. Do U.H., Sundaram M.G., Ramachandran S. and Bryant R.W.-Lipids 14: 819 (1979)

26. Woollard P.M., Hensby C.N. and Lascelles P.T.- J.Chromatogr. 166: 411 (1978)

27. Lanser A.C. and Emken E.A.- J.Chromatogr. 256: 460 (1983)

28. Meese C.O., Fischer C. and Thalheimer P.-in preparation

29. Brown C.A. and Ahuja V.K.- J.C.S.Chem. Comm. 553 (1973)

30. Brown C.A. and Ahuja V.K.- J.Org.Chem. 38: 2226 (1973)

31. Taber D.F., Phillips M.A. and Hubbard W.C.-Prostaglandins 22: 349 (1981)

32. Taber D.F., Phillips M.A. and Hubbard W.C.-Meth. Enzymol. 86: 366 (1982)

33. Candlin J.P. and Oldham A.R.- Discuss. Faraday Soc. 46: 60 (1968)

34. Schrock R.R. and Osborn J.A.- J.Am. Chem.Soc. 98: 2143 (1976)

35. Heinzman S.W. and Ganem B.- J.Am. Chem.Soc. 104: 6801 (1982)

36. Oliw E.H.- J.Chromatogr. 339: 175 (1985)

37. Axen U., Green K., Hörlin D. and Samuelsson B.- Biochem. Biophys. Res.Comm. 45: 519 (1971)

38. Green K., Granström E., Samuelsson B. and Axen U.- Anal.Biochem. 54: 434 (1973)

39. Hayashi Y., Ueda N., Yokota K., Kawamura S., Ogushi F., Yamamoto Y., Yamamoto S., Nakamura K., Yamashita K., Miyazaki H., Kato K. and Terao S.- Biochim.Biophys.Acta 750: 322(1983)

40. Hunsdiecker reaction (Br_2/CH_2Cl_2) of the silver salt of 6 (41) followed by phosphonium salt formation (Ph_3P) and saponification (47% aqueous HBr, reflux, 24h) afforded pure crystalline 7 (m.p. 204.5-206°C) in 59% overall yield from 6;

unpublished results

41. Meese C.O., Fürst O. and Borstel B.- J.Lab.Comp.Radiopharm., in press

42. Taber D.F. and Lee C.H.- J.Lab.Comp. Radiopharm. 14: 599 (1978)

43. Meese C.O., Borstel B. and Beck G.- J. Lab.Comp.Radiopharm. 19: 491 (1982)

44. Beck G. and Weimer K.E.- unpublished results

45. Corey E.J., Ohuchida S. and Hahl R.- J. Am.Chem.Soc. 106: 3875 (1984)

46. Osborn J.A., Jardine F.H., Young J.F. and Wilkinson G.- J. Chem. Soc. (A), 1711 (1966)

47. Lands W.E.M.- Ann.Rev.Physiol. 41: 633 (1979)

48. Yamamoto S.- Prostaglandins and Related Substances (Eds.C.Pace-Asciak and E.Granström), Elsevier Sci.Publ.,p.171 (1983)

49. Hamberg M. and Samuelsson B. J.Biol.Chem. 246: 1073 (1971)

50. Hamberg M. and Samuelsson B.- J.Biol. Chem. 247: 3495 (1972)

51. Oates J.A., Sweetman B.J., Green K. and Samuelsson B.- Anal. Biochem. 74: 546 (1976)

52. Abdel-Halim M.S., Hamberg M., Sjöquist B. and Änggard E.- Prostaglandins 14: 633 (1977)

53. Powell W.S.- Anal.Biochem. 128: 93 (1983)

54. Claeys M., van Hove C., Duchateau A. and Herman A.G.- Prostaglandins, Prostacyclin, and Thromboxanes Measurement (Eds. J.M. Boeynaems and A.G. Merman), Martinus Nijhoff Publ., The Hague, p.105 (1980)

55. Pace-Asciak C.- J.Am.Chem.Soc. 98: 2348 (1976)

56. Pace-Asciak C. and Nashat M.- Biochem. Biophys.Acta 487: 495 (1977)

57. Hamberg M., Svensson J. and Samuelsson B.-Proc.Nat.Acad.Sci.USA 71: 3824 (1974)

58. Svensson J.- Prostaglandins 17: 351 (1979)

59. Barrow S.E., Heavey D.J., Ennis M., Chappell C.G., Blair I.A. and Dollery C.T.- Prostaglandins 28: 743 (1984)

60. Roberts S.M. and Scheinmann F.- New Synthetic Routes to Prostaglandins and Thromboxanes, Academic Press, London (1982)

61. Corey E.J., Schaaf T.K., Huber W., Koelliker U. and Weinshenker N.M.- J.Am. Chem.Soc. 92: 397 (1970)

62. Bundy G.L., Lin C.H. and Sih J.C.- Tetrahedron 37: 4419 (1981)

63. Schaaf T.K.- Prostaglandins 18: 773 (1979)

64. Fischer C. and Meese C.O.- Biomed.Mass Spectrom. 12: 399 (1985)

65. Meese C.O.- Tetrahedron Lett. 25: 2199 (1984)

66. Meese C.O. and Frölich J.C.- Stable Isotopes-Analytical Chemistry Symposia Series 11: 743 (1982)

67. Nelson N.A. and Jackson R.W.- Tetrahedron Lett. 3275 (1976)

68. Corey E.J., Shibasaki M. and Knolle J.-Tetrahedron Lett. 1625 (1977)

69. Hanessian S. and Lavallee P.- Can.J.Chem. 59: 870(1981)

70. Meese C.O., Fürst O. and Thiem J.- 29. IUPAC Congr.Cologne (FRG), 5-10 June 1983, P 137

71. Meese C.O., Fürst O. and Fischer C.- International Symposium on Organic Chemistry of Medicinal Natural Products (IUPAC) Nov.10-14 (1985), Shanghai (China), P.No.333

72. Fürst O., Meese C.O. and Thiem J.- unpublished results

73. Maas R.L., Taber D.F. and Roberts L.J., II-Meth.Enzymol. 86: 592 (1982)

74. Meese C.O., Fischer C., Thalheimer P. and Fürst O.- Biomed. Mass Spectrom. 12: 554 (1985)

75. Fitzpatrick F., Gorman R., Green K., Kelly R. and Schneider W.-Thrombosis Res. 35: 121 (1984)

76. Schneider W.P. and Morge R.A.- Tetrahedron Lett. 3283 (1976)

77. Rokach J. and Adams J.- Acc.Chem.Res. 18: 87 (1985)

78. Scheinmann F. and Ackroyd J.- Leukotriene Synthesis: A New Class of Biologically Actice Compounds Including SRS-A, Raven Press, New York, 1984

79. Russell S.W. and Pabon H.J.J.- J.C.S.Perkin I, 545 (1982)

80. Boeynaems J.M., Brash A.R., Oates J.A. and Hubbard W.C.- Anal. Biochem. 104: 259 (1980)

81. Corey E.J. and Eckrich T.M.- Tetrahedron Lett. 25: 2419 (1984)

82. Corey E.J. and Park H.- J.Am.Chem.Soc. 104: 1750 (1982)

83. Corey E.J. and Munroe J.E.- J.Am.Chem. Soc. 104: 1752 (1982)

84. Cohen N., Banner B.L., Lopresti R.J., Wong F., Rosenberger M., Lin Y.-Y., Thom E. and Liebman A.A.- J.Am.Chem.Soc. 105: 3661 (1983)

85. Taub D., Zelawski Z.S. and Wendler N.L.-Tetrahedron Lett. 3667 (1975)

86. Rosegay A. and Taub D.- Prostaglandins 12: 785 (1976)

87. Hubbard W.C.- Meth. Enzym. 86: 571 (1982)

88. Brash A.R., Baillie T.A., Clare R.A. and Draffan G.H.- Biochem.Med. 16: 77 (1976)

89. Falardeau P., Oates J.A. and Brash A.R.-Anal.Biochem. 115: 359 (1981)

90. Falardeau P. and Brash A.R.- Meth.Enzym. 86: 585 (1982)

91. Brash A.R.- Meth.Enzym. 86: 579 (1982)

92. Lehmann W.D.- Stable Isotopes-Analytical Chemistry Symposia Series 11: 635 (1982)

93. Bollingen P. and Krieger M.- US Patent 4, 259, 523 (March 31,1981)

94. Andrist A.H. and Graas J.E.- Prostaglandins 18: 631 (1979)

95. Megargle R.G., Slivon L.E., Graas J.E. and Andrist A.H.- Anal. Chim. Acta 120: 193 (1980)

96. Green K. and Steffenrud S.- Anal. Biochem. 76: 606 (1976)

97. Nelson N.A., Jackson R.W. and Sebek O.K.-Prostaglandins 16: 85 (1978)

98. Sun F.F., Taylor B.M., Lincoln F.H. and Sebek O.K.- Prostaglandins 20: 729 (1980)

99. Pickett W.C. and Murphy R.C.- Anal. Biochem. 111: 115 (1981)

100. Murphy R.C. and Clay K.L.- Meth. Enzym. 86: 547 (1982)

101. Strife R.J., Clay K.L. and Murphy R.C.-Proceedings Int.Symp. Kansas City, MO, USA, 6-11 June 1983, (Eds.W.P.Duncan and A.B.Susan), Elsevier Sci.Publ., Amsterdam, p.301 (1983)

102. Strife R.J. and Murphy R.C.- Prostaglandins Leukotrienes Med. 13: 1(1984)

103. Murphy R.C.- Prostaglandins 28: 597 (1984)

104. McCloskey J.A., Stillwell R.N. and Lawson A.M.- Anal.Chem. 40: 233 (1968)

105. Samuelsson B., Hamberg M. and Sweeley C.C.- Anal.Biochem. 38: 301 (1970)

106. Hamberg M.- Biochem.Biophys.Res.Comm. 49, 720(1972)

107. Seyberth H.W., Sweetman B.J., Frölich J.C. and Oates J.A.- Prostaglandins 11: 381 (1976)

108. Pace-Asciak C.R. and Edwards N.S.- Meth. Enzym. 86: 552 (1982)

109. Maas R.L., Lawson J.A., Brash A.R. and Oates J.A.- Adv.Prostagland. Thromboxane Res. 229 (1983)

110. Crawford B.L.,Jr., Fletcher W.H. and Ramsey D.A.- J.Chem.Phys. 19: 406(1951)

111. DeMore W.B., Pritchard H.O. and Davidson N.- J.Am.Chem.Soc. 81: 5874 (1959)

112. Gillett P.G., Kinch R.A.H., Wolfe L.S. and Pace-Asciak C.- Am. J. Obstet. Gynecol. 112: 330 (1973)

113. Roberts L.J., II-Meth.Enzym. 86: 559 (1982)

114. Meese C.O.- J.Lab.Comp.Radiopharm. 20: 1047 (1983)

115. Fischer C. and Meese C.O.- in preparation

116. Rakoff H.- Progr.Lipid Res. 21: 225 (1982)

117. Björkhem I.- CRC Critical Reviews Clin.Lab.Sci. 11: 53(1979)

118. Murphy P.J. and Sullivan H.R.- Ann. Rev. Pharmacol. Toxicol. 20: 609(1980)