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SYNTHESIS OF ANALOGUES OF 4-DEOXYPODOPHYLLOTOXIN

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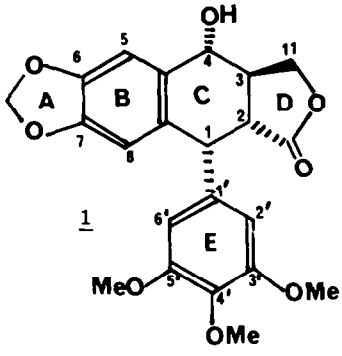
(*Received in UK* 15 *July* 1987)

Abstract - Reaction of 5,6-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-1,3-dihydrobenzo[c]thiophene-2,2-dioxide with 2-substituted maleic anhydrides at elevated temperatures provided 6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-2-substituted-tetrahydro-2,3-naphthoic anydrides. These were reduced regioselectively to provide cis-lactones, and in the case of the 2-methyl-adduct, conversion into a trans-lactone was accomplished. These compounds are formally analogues of 4-deoxypodophyllotoxin, and were assessed for their anti-cancer activity. With these and related compounds it was possible to determine the conformational preference of the molecules using n.m.r. data. This may be of importance for structure-activity correlations.

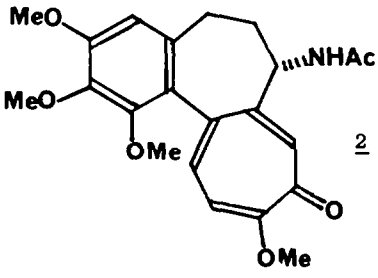
The use of plant extracts containing podophyllotoxin, 1, for medicinal purposes can be traced back over 1000 years. An early English medical text, the Leech Book of Bald, dating from AD 900-950, mentions the use of root extracts from wild chervil (which contains several podophyllotoxin derivatives) as a salve for the treatment of cancer.[1] Some 500 years later natives of the Himalayas and the North American Indians were using root extracts of the local Podophyllum perennials (P. emodi Wallach and P.peltatum Linnaeus respectively) as a treatment for warts amongst other things. Contemporary interest was first stimulated by a report that the venereal wart Condyloma acuminatum could be effectively treated with topical applications of podophyllum resin.[2] Subsequent investigations showed that extracts of P.peltatum inhibited the growth of experimental cancer cells in animals.[3]

Podophyllotoxin and the majority of its active derivatives exert their biological effects by means of a rapid, reversible binding to the protein tubulin, and subsequent inhibition of tubulin polymerisation. This polymerisation is a key feature of cell-spindle assembly during mitosis, and in consequence podophyllotoxin is an effective mitotic inhibitor.[4] This mode of action is similar to that of colchicine, 2, and the two probably bind to overlapping but non-identical sites on tubulin.

N.m.r. studies have shown that in solution podophyllotoxin (and many of its derivatives) has a favoured conformation in which the pendant aryl ring (E ring) is perpendicular (quasi-axial) to the rest of the system (ABCD rings).[5] Epimerisation occurs at pH's slightly above 7 and produces picrophyllotoxin, 3, which



1



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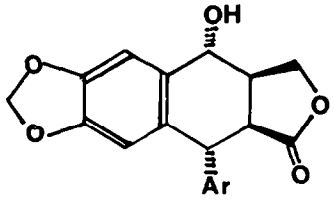
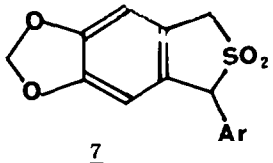


3 Ar = 3,4,5-trimethoxyphenyl

SCHEME



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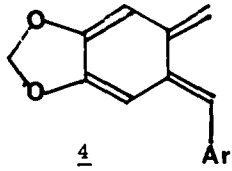

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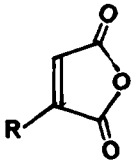
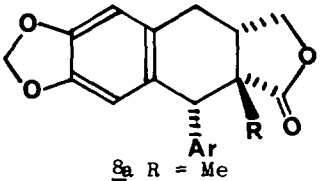
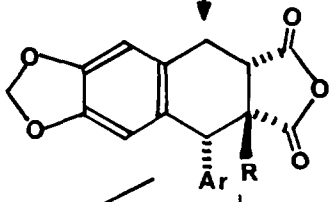


5a R=Me
5b R=CF_3
5c R=Ph



8a R = Me
8b R = CF_3
8c R = Ph

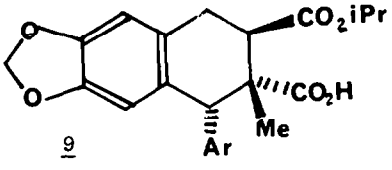
K-selectride



6a R = Me
6b R = CF_3
6c R = Ph

$Ph_3P=CHCO_2Me$

xs.sod.isopropoxide



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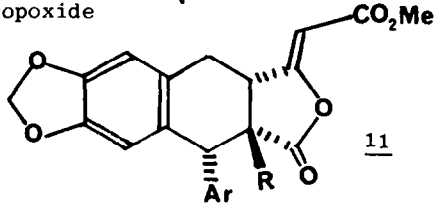
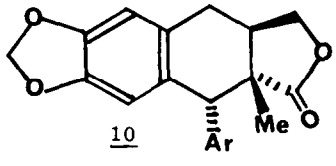


11

$LiAlH_4$
then
DCC

H_2/Pd



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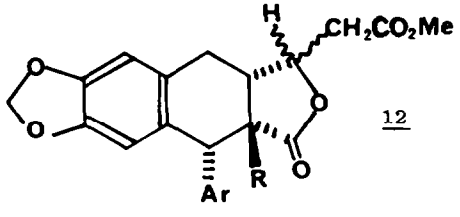


12

is essentially devoid of biological activity. This lignan has a favoured conformation in solution in which the pendant aryl substituent is roughly coplanar with the ABCD ring system, and it is thus tempting to conclude that the conformation adopted by podophyllotoxin and derivatives is essential for biological activity.

In order to explore this phenomenon further, we have prepared a number of analogues of 4-deoxypodophyllotoxin which possess both cis- and trans-lactone rings, and in which epimerisation is precluded due to the presence of a 2-substituent. Our route to these analogues is shown in the Scheme and involves cycloaddition reactions of the orthoquinodimethane, 4, with 2-substituted maleic anhydrides, 5, to produce 1-(3,4,5-trimethoxyphenyl)-tetrahydronaphthoic anhydrides, 6. The orthoquinodimethane was prepared in situ by heating the sulphone, 1-(3,4,5-trimethoxyphenyl)-5,6-methylenedioxy-1,3-dihydrobenzo[c]thiophene-2,2-dioxide, 7, at 200°C. Synthesis of the sulphone and some of its reactions have been described previously.[6] The cycloadducts were reduced regioselectively using K-selectride (potassium tri-sec.butyl borohydride) to provide good yields of the cis-lactones, 8. Alternatively, in the case of the 2-methyl cycloadduct, 6a, treatment with hot sodium isopropoxide in isopropanol produced the epimerised ester acid, 9, which could be reduced regioselectively with lithium aluminium hydride, and subsequently dehydrated with DCC to yield the trans-lactone, 10, 2-methyl,4-deoxypodophyllotoxin. In addition treatment of 6a with carbomethoxymethylidene triphenylphosphorane in benzene provided the Wittig product, 11, as the major product (52% yield after chromatography and recrystallisation). Catalytic hydrogenation provided a major product, 12a, and a minor product, 12b. Essential proton n.m.r. data for all of these compounds and for 4-deoxypodophyllotoxin is presented in the Table.

Table

compound	1-H	3-H	4-H	5-H	8-H	2',6'-H	11-H
4-deoxy-1	4.60	2.72	2.78,3.07	6.66	6.51	6.34	3.92,4.45
8a	3.93	2.98	2.65-2.71	6.71	6.59	6.41	3.27,4.25
8b	4.51	3.33	2.82,3.13	6.70	6.55	6.39	3.60,4.40
8c	4.48	3.45	2.87,3.29	6.73	6.39	6.65	3.81,4.33
10	4.25	2.67-2.96		6.66	6.48	6.34	3.90,4.32
12a	3.86	2.85	2.32,2.60	6.72	6.58	6.92	5.10
12b	4.00	2.40	2.90	6.74	6.64	6.36	4.00

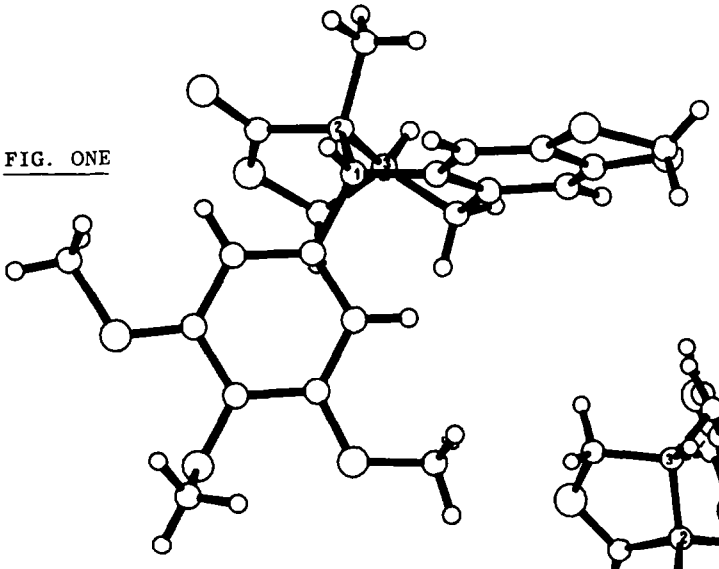
The most notable feature is the downfield shift of protons 2'-H and 6'-H observed for compounds 8c and 12a. When X-ray studies were carried out on the lactones 8 and 10, all except 8c were shown to possess essentially planar ABCD ring systems with the pendant aryl ring and 2-substituent axially disposed. In contrast, 8c had both pendant aryl and phenyl groups quasi-equatorially disposed. The structures are shown in Figures One to Four.[7] Further, although no X-ray structural data was obtained for the lactones, 12, the minor component could be converted into the major component upon treatment with base. It is likely that the most stable isomer is the one with all substituents held in a quasi-equatorial arrangement, and the downfield shift observed for 12a would be in accord with this.

When assessed for biological activity,[8] in vivo against the P388 mouse leukaemia, all three cis-lactones, 8, possessed modest but reproducible activity: extension of lifetime by up to 30% at a dose-rate as low as 36mg/kg administered over five consecutive days. The trans-lactone, 10, exhibited no in vivo

activity, despite having the correct conformation, and this may be due to its very poor solubility in aqueous DMSO (administration solvent), or to rapid metabolism under physiological conditions. Interestingly, Durst et al. found that 2-methylpodophyllotoxin was biologically inactive, though the corresponding 2-chloro-analogue was found to display significant anti-tumour activity (56% extension of lifetime at 40mg/Kg on days 1 and 5).[9] The activity displayed by the 2-phenyl-lactone, 8c, is remarkable given that in both the solid state and in $CDCl_3$ solution it adopts the conformation that is usually associated with lack of biological activity, and other factors may need to be considered. However, it is clear that n.m.r. may be used to predict the favoured conformations of podophyllin analogues, and that a trans-lactone, as found in podophyllotoxin, is not an absolute requirement for anti-tumour activity.

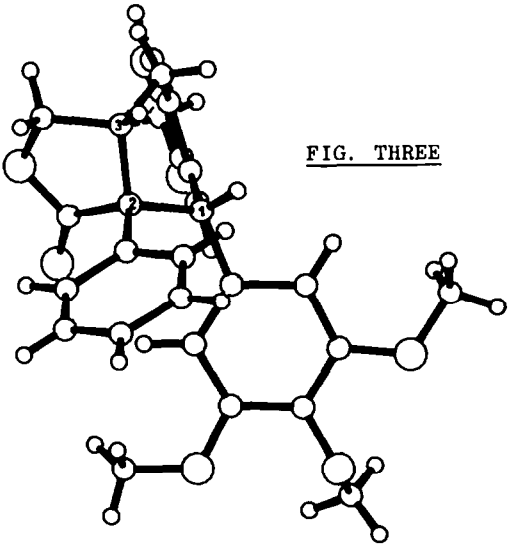
Finally, some comment concerning the regioselectivity observed in these cycloadditions is required. The 2-trifluoromethyl- and 2-phenyl-adducts (8b and 8c) were not accompanied by other products, and this regio-selectivity would be predicted by FMO theory.[10] The 2-methyl-cycloadduct, 8a, was formed together with the 3-methyl-isomer (ratio 7:4), and this must reflect electronic (inductive) contributions by the methyl group which render the C-4 carbonyl of citraconic anhydride (5a) less electron deficient than the C-1 carbonyl (cf. ref.10, page 137). The actual cycloadditions thus proceed in fair yield with predictable regio- and stereochemical outcome, and a wide range of podophyllin analogues are now accessible for further biological investigations.

FIG. ONE



8a

FIG. THREE



8c

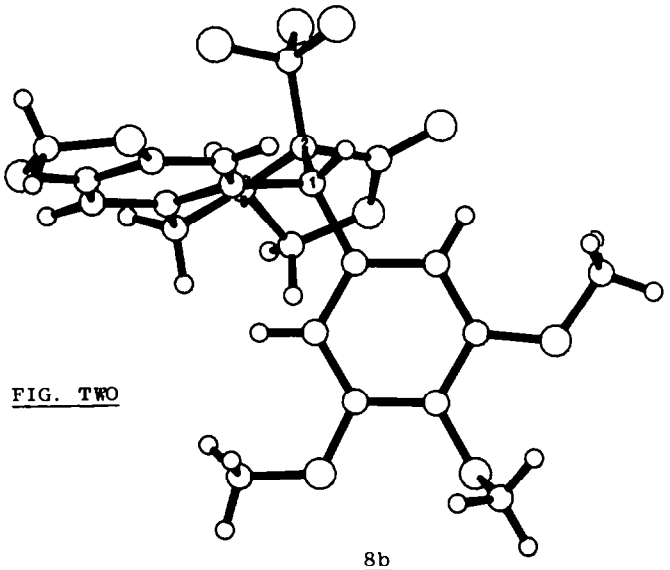


FIG. TWO

8b

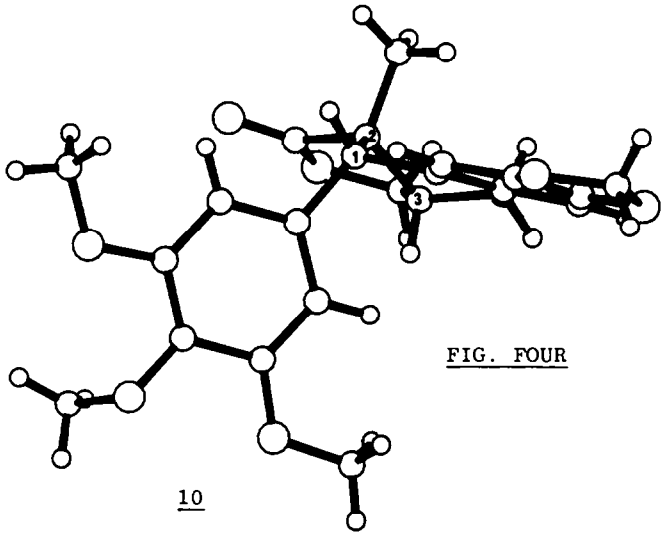


FIG. FOUR

10

Experimental

I.r. spectra were recorded with a Perkin-Elmer 157 double beam grating spectrophotometer (all samples were dissolved in CH_2Cl_2): n.m.r. spectra were recorded with a Perkin-Elmer R34 (220MHz) instrument, or with a Bruker WM400 (400MHz) instrument (University of Warwick) using tetramethylsilane as internal standard; flash chromatography was performed using Merck silica gel (250-400 mesh); solvents were distilled from calcium hydride when required anhydrous; and pet. ether means the fraction boiling between 40 and 60°C.

Cycloadditions with orthoquinodimethane, 4.

The sulphone, 5, and the appropriate dienophile (1-4 equivalents) were added to di-n-butyl phthalate (2ml per mmole of sulphone), and the mixture placed under nitrogen prior to heating to 160-200°C. After ca. 3 hours, the mixture was allowed to cool, then loaded directly onto a column of silica gel, and the components separated by flash chromatography.

1-(3,4,5-trimethoxyphenyl)-2-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydro-2,3-naphthoic anhydride, 6a.

This cycloadduct was obtained together with the 3-methyl-regioisomer in a combined yield of 90%, and a ratio of 7:3. M.pt. 153°C (from ether:pet. ether); ν_{max} (dichloromethane) 2900, 1850, and 1785 cm^{-1}; $\delta(CDCl_3)$ 1.42 (3H,s,Me), 3.10 (1H,dd,J 9.8 and 15.9Hz, H-4$_\alpha$), 3.18 (1H,dd,H-3), 3.40 (1H,dd,J 3.2 and 15.9Hz,H-4β), 3.75 (6H,s,3',5'-OMe), 3.80 (3H,s,4'-OMe), 3.90 (1H,s,H-1), 5.90 (2H,s,methylenedioxy), 6.32 (2H,s,H-2',H-6'), 6.58 (1H,s,H-8), and 6.75 (1H,s,H-5) ppm; found C 64.95%, H 5.23%; $C_{23}H_{22}O_8$ requires C 64.77%, H 5.20%.

1-(3,4,5-trimethoxyphenyl)-2-trifluoromethyl-6,7-methylenedioxy-1,2,3,4-tetrahydronaphthoic anhydride, 6b

The cycloadduct was obtained in 61% yield after chromatography and recrystallisation: no trace of the alternative regioisomer was isolated. M.pt. 181-183°C; ν_{max} (dichloromethane) 3050, 2980, 1860, 1793, 1590, 1487, 1463, 1420, 1327, 1240, 1230, 1195, 1060, 1040, 1000, 960, 940, 895, and 865 cm^{-1}; δ ($CDCl_3$) 3.4-3.6 (3H,m, H-3 and H-4), 3.8 (6H,s,3',5'-OMe), 3.86 (3H,s,4'OMe), 4.6 (1H,s,H-1), 6.0 (2H,s,methylenedioxy), 6.35 (2H,s,H-2' and H-6'), 6.63 (1H,s,H-8), 6.84 (1H,s,H-5) ppm; found C 57.94%, H 4.11%; $C_{23}H_{19}F_3O_8$ requires C 57.50%, H 3.99%.

1-(3,4,5-trimethoxyphenyl)-2-phenyl-6,7-methylenedioxy-1,2,3,4-tetrahydro-2,3-naphthoic anhydride, 6c.

The cycloadduct was obtained as the sole regioisomer in 65% yield after chromatography. M.pt. 164-165°C; ν_{max}(dichloromethane) 3060, 2990, 1850, 1785, 1590, 1510, 1490, 1470, 1425, 1333, 1130, 1060, 960, 945, 925, and 870 cm^{-1}; δ ($CDCl_3$) 3.45-3.60 (2H,m,4-H$_\alpha$ and 4-H$_\beta$), 3.7 (6H,s,3',5'-OMe), 3.8 (3H,s,4'OMe), 3.80-4.05 (1H,m,H-3), 4.40 (1H,s,H-1), 5.85 (2H,s,methylenedioxy), 6.30 (2H,s, 2H-2' and H-6'), 6.45 (1H,s,H-8), 6.75 (1H,s,H-5), and 7.2-7.5 (5H,m,phenyl)ppm; found C 68.70%, H 4.46%; $C_{28}H_{24}O_8$ requires C 68.83%, H 4.54%.

Reductions of Anhydrides, 6.

The anhydride (1 equiv.) in dry THF (30 ml per mmole) was placed under nitrogen at -78°C, and treated with K-selectride (1.0M in THF, 2 equiv.). Reaction was normally complete after 4-5 hours and after addition of water (ca. 1 ml per mmole), the mixture was allowed to warm to RT. Sodium hydroxide solution (2M, 5 ml per mmole) and hydrogen peroxide (30%, 3 ml per mmole) were added, and

the mixture stirred overnight. Hydrochloric acid (6M, 3 ml per mmole) was then added, and the mixture stirred at RT for 15 minutes, before transfer to a separating funnel. The organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were treated with p-toluene sulphonic acid (0.5g.), and the solution was stirred overnight at RT. After concentration, flash chromatography provided pure samples of the cis-lactones, 8, in yields of around 60-70%.

2-Methylisodeoxypicrophyllotoxin, 8a.

M.pt. 108°C (from ether/pet.ether); ν_{max}(dichloromethane) 2900, 1765, 1590, 1505, 1485, 1330, 1230, 1125, and 1040 cm^{-1}; δ ($CDCl_3$) 1.19 (3H,s,Me), 2.65-2.71 (2H,m,H-4), 2.91-3.05 (1H,m,H-3), 3.27 (1H,t,J 8.5Hz,H-11_β), 3.71 (6H,s,3',5'-OMe), 3.77 (3H,s,4'-OMe), 3.93 (1H,s,H-1), 4.25 (1H,t,J 8.5Hz, H-11_α), 5.92 (2H,s,methylenedioxy), 6.41 (2H,s,H-2' and H-6'), 6.59 (1H,s,H-8), 6.71 (1H,s,H-5)ppm; found C 66.97%, H 5.82%; $C_{23}H_{24}O_7$ requires C 66.97%, H 5.87%.

2-trifluoromethylisodeoxypicrophyllotoxin, 8b.

M.pt. 171-173°C (from dichloromethane/ether); ν_{max} (CH_2Cl_2) 3060, 2990, 1783, 1590, 1510, 1490, 1465, 1420, 1390, 1330, 1240, 1190, 1170, 1130, 1040, 1005, 940, 900 cm^{-1}; δ ($CDCl_3$) 2.82 (1H,dd,J 4.5 and 16.5Hz,H-4_β), 3.13 (1H,dd,J 8.5 and 16.5Hz,H-4_α), 3.33 (1H, m, J 4.6,8.9,9.0Hz,H-3), 3.60 (1H,dd,J 9.0 and 9.0Hz,H-11_β), 3.75 (6H,s,3',5'-OMe), 3.82 (3H,s,4'-OMe), 4.40 (1H,dd,J 9.0 and 9.0Hz,H-11_α), 4.51 (1H,s,H-1), 5.95 (2H,2xs,methylenedioxy), 6.39 (2H,s,H-2' and H-6'), 6.55 (1H,s,H-8), 6.70 (1H,s,H-5) ppm; found C 58.61%, H 4.46%; $C_{23}H_{21}F_3O_7$ requires C 59.21%, H 4.54%.

2-Phenylisodeoxypicrophyllotoxin, 8c

M.pt. 248-249°C; ν_{max} (dichloromethane) 3060, 2995, 1770, 1590, 1485, 1470, 1425, 1335, 1245, 1180, 1130, 1045, 1010, 945, 900 cm^{-1}; δ($CDCl_3$) 2.87 (1H,dd,J 2.5 and 16.0Hz,H-4_β), 3.29 (1H,dd,J 9.0 and 16.0Hz,H-4_α), 3.45 (1H,m,H-3), 3.73 (6H,s,3',5'-OMe), 3.80 (3H,s,4'-OMe), 3.81 (1H,dd,J 5.2 and 9.0Hz,H-11_β), 4.33 (1H,dd,J 7.5 and 9.0Hz,H-11_α), 4.48 (1H,s,H-1), 5.89 (2H,s,methylenedioxy), 6.39 (1H,s,H-8), 6.65 (2H,s,H-2' and H-6'), 6.73 (1H,s,H-5), 7.13-7.39 (5H,m, phenyl-H) ppm; found C 70.56%, H 5.54%; $C_{28}H_{26}O_7$ requires C 70.86%, H 5.52%.

2-Methyldeoxypodophyllotoxin, 10

The anhydride 6a (3.76g, 8.8mmole) in isopropanol (200 ml) was added to excess sodium isopropoxide in isopropanol (100 ml), and the mixture was heated at reflux for 5 hours, then stirred overnight at 30°C. After cooling and acidification, the isopropanol was removed under reduced pressure, and the ester acid, 9, was extracted into dichloromethane. The organic extract was dried, and concentrated to an oil prior to treatment with excess $LiBH_4$solution (2M in THF, 28 ml) under nitrogen, and at reflux. Upon completion of the reduction,dilute HCl was added to the mixture, held at 0°C, and the resultant hydroxy acid was extracted into dichloromethane. After drying and concentrating the extract, the product was dissolved in THF (40 ml), and treated with dicyclohexylcarbodiimide (0.5g). The solution was stirred at RT overnight, and then refluxed for 3 hours. The product was then purified by flash chromatography eluting with ethyl acetate: pet.ether (1:1) to yield trans-lactone (10) (0.47g, 13% overall from 6a).
M.pt. 210°C (from $CHCl_3$/pet.ether): ν_{max} (CH_2Cl_2) 3000-2820, 1778, 1590, 1505, 1490, 1470, 1330, 1235, 1125, 1040, and 1000 cm^{-1}; δ($CDCl_3$) 1.25 (3H,s,Me), 2.67-2.74 (1H,dd,J 16 and 12Hz, H-4_β), 2.84-2.96 (2H,m,H-3 and H-4_α),

3.72 (6H,s,3',5'-OMe), 3.78 (3H,s,4'-OMe), 3.90 (1H,dd,J 12 and 8Hz,H-11$_\beta$), 4.25 (1H,s,H-1), 4.32 (1H,dd,J 12 and 9Hz,H-11$_\alpha$), 5.92 (2H,s,methylenedioxy), 6.34 (2H,s,H-2' and H-6'), 6.48 (1H,s,H-8), 6.66 (s,1H,H-5) ppm; found C 66.98%, H 6.16%; $C_{23}H_{24}O_7$ requires C 66.97%, H 5.87%.

11-Carbomethoxymethylidene-2-methylisodeoxypicrophyllotoxin, 11

To a solution of anhydride 6a (1.413g, 3.3mmole) in CH_2Cl_2 (100 ml) was added carbomethoxymethylidene triphenylphosphorane (2.2g, 6.6mmole), and the clear solution was then stirred at RT for five days. Concentration and subsequent flash chromatography, eluting with ether:pet.ether (2:1) yielded white crystals of the unsaturated ester, 11 (0.835g, 52% yield). M.pt. 220°C; ν_{max}(CH_2Cl_2) 2840, 1815, 1720, 1660, 1590, 1505, 1485, 1460, 1370, 1330, 1240, 1180, 1130, 1100, 1040, 975, and 940 cm^{-1}; δ($CDCl_3$) 1.34 (3H,s,Me), 3.12-3.26 (2H,m,J 16.8, and 2.5Hz,H-4$_\alpha$ and H-4$_\beta$), 3.78 (4H,ester OMe,H-3), 3.86 (6H,s,3',5'-OMe), 3.90 (3H,s,4'-OMe), 4.00 (1H,s,H-1), 5.68 (1H,d,J 2Hz, olefinic-H), 5.88 (2H,s,methylenedioxy), 6.55 (1H,s,H-8), 6.68 (1H,s,H-5), 7.05 (2H,s,H-2' and H-6') ppm; found C 64.81%, H 5.40%; $C_{26}H_{26}O_9$ requires C 64.71%, H 5.43%.

Hydrogenation of 11

The unsaturated ester, 11 (0.49g, 1.02mmole) was dissolved in ethyl acetate (80 ml), and hydrogenated using 5% palladium on charcoal. Two products were formed with R_F 0.25 (major product) and R_F 0.34 (minor product) on tlc using ether:pet.ether (4:1) as elutant. Flash chromatography using the same system yielded the minor product, 12b (0.092g, 19%), and the major product, 12a (0.20g, 41%).

Minor Product, 12b: m.pt. 169°C (from ether:pet.ether); ν_{max} (CH_2Cl_2) 2920, 1770, 1745, 1590, 1505, 1485, 1460, 1330, 1225, 1130, and 1040 cm^{-1}; δ ($CDCl_3$) 1.24 (3H,s,Me), 2.40 (1H,m,H-3), 2.60 (2H,dd,J11 and 6Hz,CH_2CO_2Me), 2.90 (2H,m,H-4$_\alpha$ and H-4$_\beta$), 3.66 (3H,s,ester OMe), 3.74 (6H,s,3',5'-OMe), 3.82 (3H,s,4'-OMe), 4.0 (2H,m,H-11 and H-1), 5.95 (2H,s,methylenedioxy), 6.36 (2H,s,H-2' and H-6'), 6.64 (1H,s,H-8), 6.74 (1H,s,H-5) ppm; found C 64.37%, H 5.93%; $C_{26}H_{28}O_9$ requires C 64.40%, H 5.83%.

Major Product, 12a: m.pt. 179°C (from ether:pet.ether); ν_{max} (CH_2Cl_2) 2920, 1770, 1740, 1590, 1505, 1480, 1460, 1330, 1125, and 1040 cm^{-1}; δ ($CDCl_3$) 1.32 (3H,s,Me), 2.30-2.60 (2H,dd,J 14 and 7Hz,H-4$_\alpha$ and H-4$_\beta$), 2.85 (1H,m,H-3), 3.71 (3H,s,CO_2Me), 3.82 (6H,s,3',5'-OMe), 3.86 (4H,s,4'-OMe and H-1), 5.10 (1H,q, J 7Hz,H-11), 5.90 (2H,s,methylenedioxy), 6.58 (1H,s,H-8), 6.72 (1H,s,H-5), 6.92 (2H,s,H-2' and H-6') ppm; found C 64.46%, H 5.81%; $C_{26}H_{28}O_9$ requires C 64.40%, H 5.83%.

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