Forskolin and 7-Deacetylforskolin: Study of the Reactivity Behaviour towards Different Phosphorylating Agents

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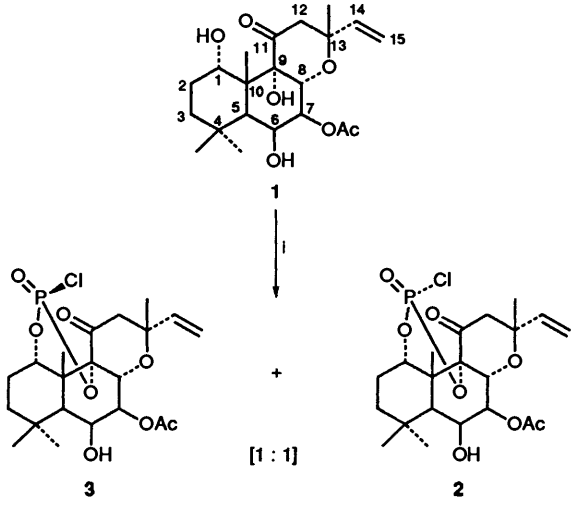
Reaction of different phosphorylating agents such as $POCl_3$, bis-(*p*-methoxyphenyl) phosphorochloridate, 2-chloro-2-oxo-1,3,2-dioxaphospholane and bis-(trifluoroethyl) phosphite with forskolin gave the corresponding isomers α- and β-1, 9-cyclic phosphates/phosphites. 1,2-Dibromo-2-phenylethylphosphonic acid reacted with forskolin and 7-deacetylforskolin to give forskolin 1-phosphate **17** and 7-deacetylforskolin 7-phosphate **18**, respectively.

Forskolin, a labdane diterpenoid isolated from *Coleus forskohlii*,[1] shows a number of interesting biological properties, such as positive inotropic, antihypertensive, lowering of intraocular pressure and adenylate cyclase activator activities.[2] A large amount of chemical[2a] and biological[2b] data has been accumulated on forskolin and deoxyforskolin.

Forskolin is a polyhydroxylated labdane, and most of the hydroxy groups have been substituted with the aim of studying structure–activity relationships (SAR).[3a] The relative reactivity of different hydroxy groups based on their steric hindrance has, to a large extent, been delineated by different workers.[3b] The hydroxy group at C-7 for 7-deacetylforskolin is the most approachable; next available is the hydroxy group at C-1, followed by the highly hindered hydroxy group at C-6. We considered it a challenge and a useful exercise to try to phosphorylate specific hydroxy group/groups one at a time. Some of the resulting phosphates could be converted into water-soluble derivatives, a highly desirable feature for utilization of these compounds for different pharmacological studies. Forskolin by itself has very low water solubility and this has been one of the major drawbacks for the development of forskolin as a positive inotropic agent. Incidently it may also be mentioned that only a limited amount of work on phosphorylation of terpenes in general has appeared. Consequently, we have synthesized phosphate and phosphite derivatives of forskolin and 7-deacetylforskolin at positions 1 and 7 as well as cyclic phosphates at positions at 1, 9 and 6, 7, respectively. This paper describes this work.

Results and Discussion

Forskolin **1** reacted with $POCl_3$, Et_3N and pyridine in diethyl ether at 0–20 °C[3a] to give cyclic phosphorochloridates **2** and **3** (Scheme 1). The reaction is proposed to take place first at the OH group at position 1, and to be followed by cyclization through participation of the 9-OH group. It was observed that Cl groups on the new $OPOCl_2$ intermediate showed no special discrimination for attack from 9-OH, therefore both α-isomer **2** and β-isomer **3** were formed in a 1:1 mixture and were separated by flash chromatography on silica gel with MeCN–C_6H_6 (15:85) as eluent. In the absence of phosphorus NMR, we have attempted to give a *tentative* stereochemical assignment of the α-chloro **2** and β-chloro **3** based on their ^1H NMR data. In forskolin **1**, the 12α-hydrogen is deshielded[4] and appears at low field compared with the 12β-hydrogen, due to the 1α- and 9α-OH groups. Substitution on these OH groups reduces the deshielding effect on the 12α-proton, thereby narrowing the chemical-shift difference between the 12α and 12β protons, as in compound **12a**[5] (Fig. 1). In cyclic β-phosphorochloridate **3**, the 12β and 12α protons appeared at δ 2.74 and 3.16, respectively,



Scheme 1 *Reagents:* i, $POCl_3$, Et_3N, pyridine, Et_2O

leaving a difference in their chemical shifts of only 0.42 ppm. In the corresponding cyclic α-phosphorochloridate **2** no decrease in the chemical-shift difference was observed. The 12α-proton appeared at δ 3.50 and the 12β-H at δ 2.57, giving difference of 0.93 ppm in their chemical shifts. We have made an attempt to explain this phenomenon with the help of Dreiding models (Fig. 1) of the two isomers **2** and **3**. In isomer **2** the P=O bond is facing the 12α-proton, which deshields this proton and mimics the deshielding effect of the 1,9-OH groups of forskolin **1**. However, in the isomer **3** the chloro group is β-oriented and the P=O bond occupies an α-orientation. The P=O bond lies completely out of the plane of either the 12α- or the 12β-hydrogen. Therefore no special deshielding effect is observed and the 12α- and the 12β-proton appear close in their chemical shifts, as expected, because of cyclization (which we have observed when 1,9-OH groups are converted into cyclic structures such as acetonides)[5]. This phenomenon is common to all the pairs of isomers reported here and has been used as the basis for assigning the stereochemistry of different pairs of cyclic 1,9-phosphates of forskolin **1**.

Forskolin **1** reacted with bis-(*p*-methoxyphenyl) phosphorochloridate in pyridine[6] to give two cyclic phosphates, α-isomer **4** and β-isomer **5**, in 56% yield, along with the minor amounts (1.6%) of the rearranged elimination product **6**. The yield of epoxide **6** could be increased to 13% upon replacement of pyridine with ethyldiisopropylamine (Hunig's base). The *p*-methoxyphenoxy group being a rather poor leaving group, it was expected that only the chloro group would be displaced to

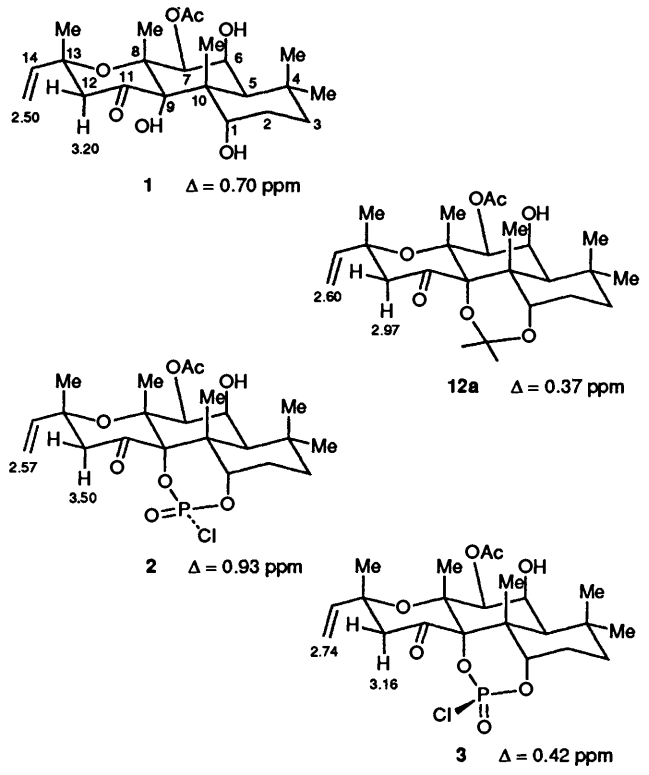


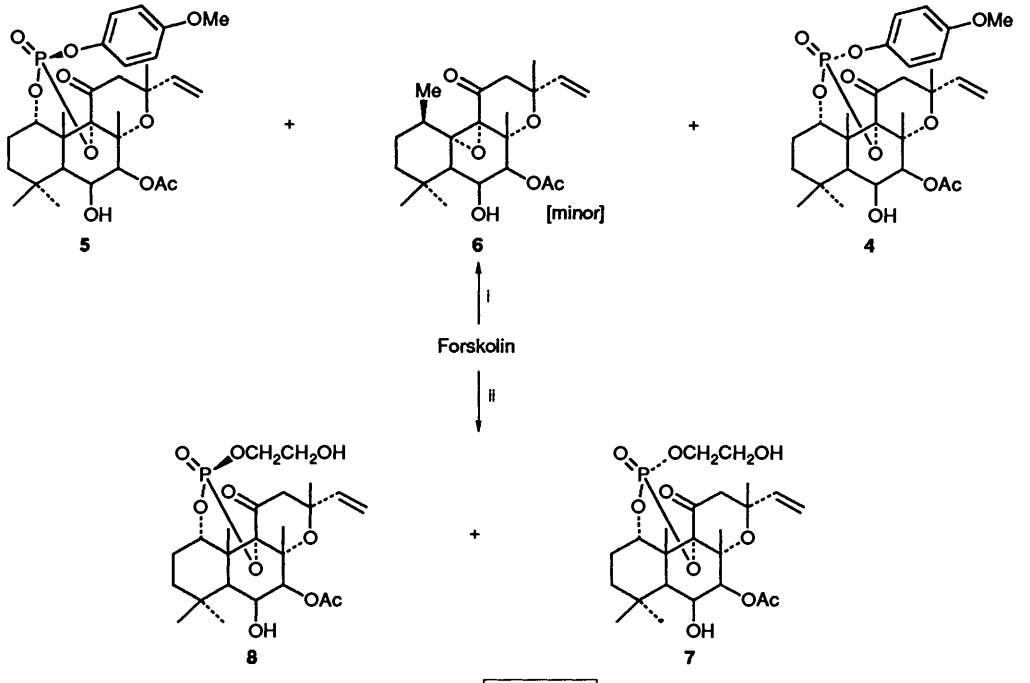
Fig. 1

yield the phosphate at position 1. However, quite surprisingly, due to internal participation of the 9-OH group, a *p*-methoxyphenoxy group was displaced, giving rise to isomers **4** and **5** (Scheme 2). The structure of the rearranged elimination product **6** was assigned with the help of spectral data, the main characteristic ^{1}H NMR peaks being: the 1-Me appeared at δ 0.74 as a doublet with J 7.11 Hz, 1-H showed up as distorted sextet at δ 1.95, which became simplified when 1-Me was irradiated, and the 5-H proton gave a doublet of 6.1 Hz, indicating a change in the conformation of ring B. This ring presumably has assumed a distorted boat shape. The dihedral angle between 5-H and 6-H has considerably narrowed, thereby explaining the observed coupling constant.

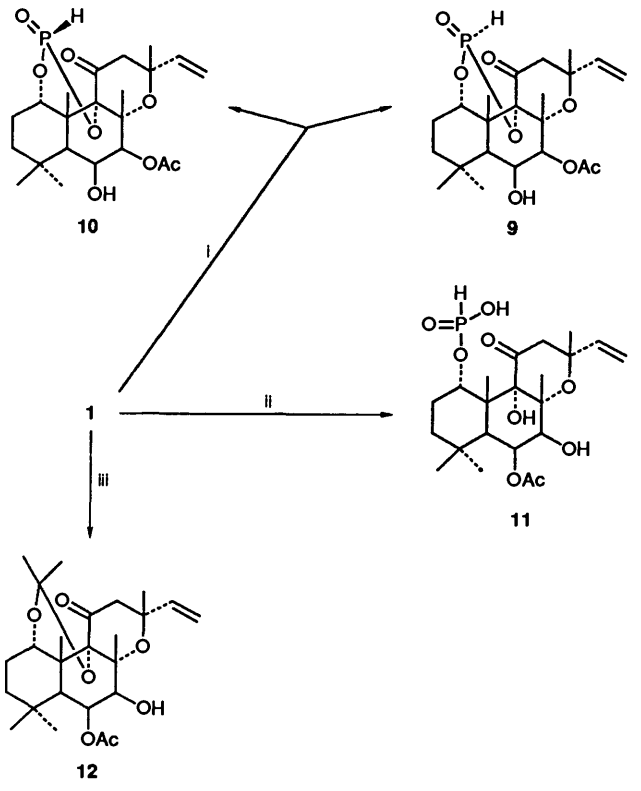
2-Chloro-2-oxo-1,3,2-dioxaphospholane[7] was allowed to react with forskolin **1** in pyridine in order to attempt reaction at position 1. However, this failed again due to internal participation of the 9-OH group and led to the formation of the corresponding cyclic phosphates, α-isomer **7** and β-isomer **8** (Scheme 2). The assignment of the isomers is again based on the relative chemical shifts of the 12β- and 12α-hydrogen (see Experimental section).

An interesting set of reactions took place when forskolin **1** reacted with bis(trifluoroethyl) hydrogen phosphite*,[8] under different conditions. In the presence of pyridine two isomeric cyclic phosphites (phosphonates), α-isomer **9** and β-isomer **10**, were isolated (Scheme 3). However, in benzene and without base the 1-mono(phosphonate) **11** was isolated as the sole product. The acetyl group at position 7 had migrated to C-6, as evidenced by its ^{1}H NMR signal. Compound **11** was isolated as its diethylamine salt. When forskolin **1** reacted with bis-(trifluoroethyl) hydrogen phosphite in acetone, again without base, no phosphorylation took place. Instead the acetonide **12** was isolated. We can offer some explanation for the formation of products **9**–**12**. In the first reaction pyridine acts both as catalyst and as solvent. The phosphorylating reagent behaves in a manner similar to those discussed earlier to give the two cyclic isomers **9** and **10**. Bis(trifluoroethyl) hydrogen phosphite reagent is acidic and in benzene condensation at C-1 is favoured. No participation by the 9-OH group is seen. Furthermore the anhydrous acidic environment can also favour the migration of the 7-acetyl group to the 6-position to give compound **11**. Finally, with acetone as the solvent, the phosphorylating reagent acts as an acid catalyst and helps acetone to react with the 1- and 9-OH groups to give the 1,9-acetonide **12** at a much faster rate compared with phosphorylation. The above three

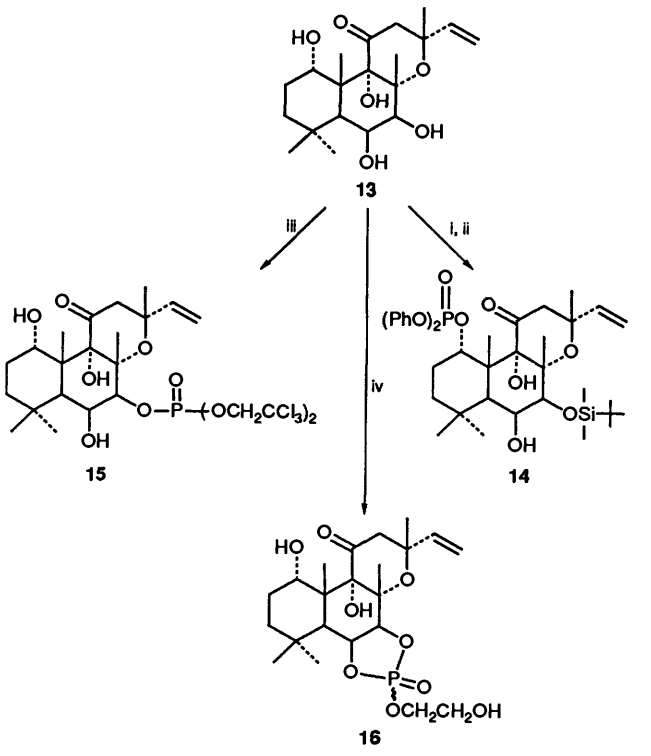
* Drawn as the tautomeric form bis(trifluoroethyl) phosphonate in Scheme 3.



Scheme 2 *Reagents:* i, $(p\text{-MeOC}_6H_4O)_2P(=O)Cl$, pyridine; ii, $Cl(O=)POCH_2CH_2O$, pyridine



Scheme 3 *Reagents:* i, $(CF_3CH_2O)_2P(=O)H$, pyridine or $ClP\overline{OCH_2CH_2O}$, pyridine; ii, $(CF_3CH_2O)P_2(=O)H$, benzene; then Zn, pyridine, water; iii, $(CF_3CH_2O)_2P(=O)H$, acetone



Scheme 4 *Reagents:* i, TBDMSCl, imidazole, DMF; ii, $(PhO)_2P(=O)Cl$, NaH; iii, $(CCl_3CH_2O)_2P(=O)Cl$, pyridine; iv, $Cl(O=)\overline{POCH_2CH_2O}$, pyridine

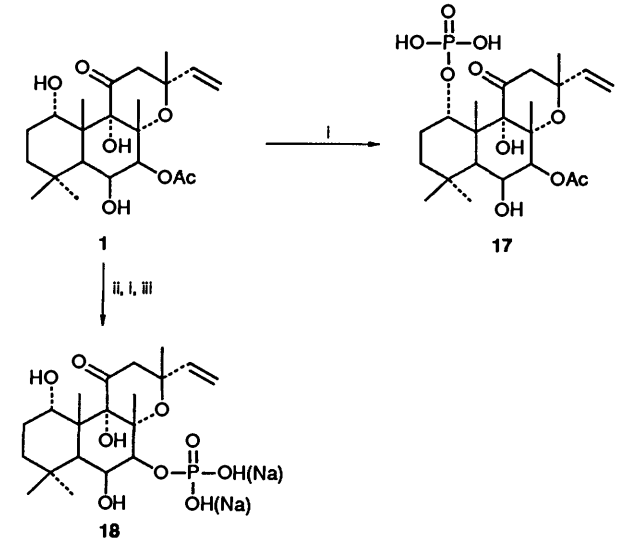
reactions clearly indicate that the phosphorylation of forskolin is sensitive to the overall reaction conditions.

Isomers **9** and **10** were treated separately with *N*-chlorosuccinimide (NCS) in C_6H_6, and the corresponding chloro compounds isolated were identical with compounds **2** and **3**, respectively. Formation of these chloro compounds proceeded with retention of their respective configuration.

Forskolin **1** was hydrolysed to 7-deacetylforskolin[1] **13** and this was allowed to react with one mole equivalent of 2-chloro-2-oxo-1,3,2-dioxaphospholane in the presence of pyridine to give 6,7-phospholane **16**. The 6- and 7-OH groups behaved in a manner similar to the 1- and 9-OH groups in compound **1**. We believe that condensation takes place at the less hindered 7-position followed by participation of the neighbouring 6-OH group. The original phospholane ring then opens up and gives the new phospholane **16**. The stereochemistry of the hydroxyethoxy group could not be determined due to overlap of the peaks in the ^1H NMR spectrum.

7-Deacetylforskolin **13**, on treatment with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in dimethylformamide (DMF)[9] gave 7-(TBDMS)deacetylforskolin, which was then treated with diphenyl phosphorochloridate[10] in NaH–tetrahydrofuran (THF), to give only compound **14** (Scheme 4), and no cyclic product was detected. Reaction of compound **13** with bis-(2,2,2-trichloroethyl) phosphorochloridate[11] in pyridine gave compound **15**. All attempts to cleave the 2,2,2-trichloroethyl group gave a mixture of products.

1,2-Dibromo-2-phenylethylphosphonic acid is reported to generate 'PO_3^-' species,[12] a controversial affair[13] which attracted our attention. We thought it would be worthwhile to attempt phosphorylation of forskolin **1** and 7-deacetylforskolin **13** with this reagent. Monophosphorylation at the 1- and 7-position, respectively, were achieved. Forskolin **1** was treated with 1,2-dibromo-2-phenylethylphosphonic acid in methylene dichloride and Hunig's base as catalyst. A relatively clean reaction took place to give the 1-phosphate **17** in 30% yield. Similarly, compound **13** reacted under identical conditions to give the corresponding 7-phosphate **18** in 29% yield (Scheme 5), which was further converted into its sodium salt by passage through Dowex 50X (Na^+). It was observed that these reactions do not go to completion and almost 60% of the starting material



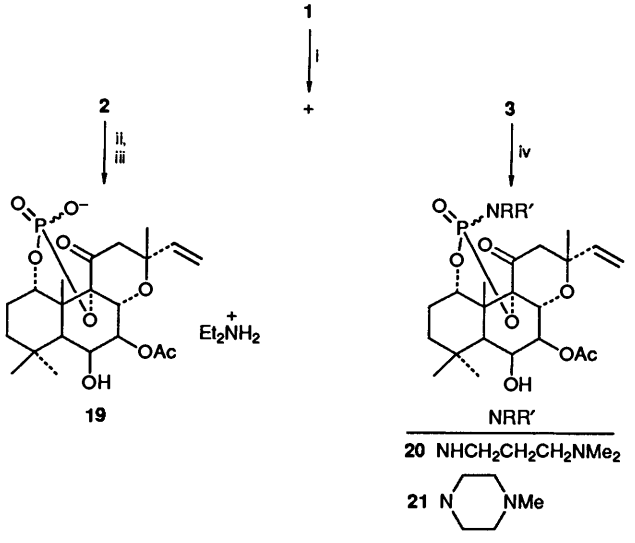
Scheme 5 *Reagents:* i, $PhCHBrCHBrP(=O)(OH)_2$, Hunig's base; ii, ^-OH; iii, Dowex (Na^+)

was recovered. We have also noticed that the above reagent phosphorylates selectively. There is no need to protect any hydroxy groups, as was demonstrated with forskolin **1** and 7-deacetylforskolin **13**.

Attempted hydrolysis of compound **2** with water–1,4-dioxane

resulted in a mixture. This may be due to release of HCl, which leads to destruction of the molecule under reflux.[14] However, using Na_2HPO_4 and KH_2PO_4 as buffer along with water–1,4-dioxane produced hydrolysed product **19** in good yield. It was isolated as the diethylamine salt. The relative stereochemistry of the new OH group could not be determined by ^1H NMR spectroscopy because both OH and P=O groups can impart a deshielding effect on protons at C-12. More important is that presumably these are resonance structures. The chemical-shift difference between the 12α- and 12β-proton is 1.39 ppm.

Condensation of β-chloro isomer **3** with 3-(dimethylamino)propylamine and with *N*-methylpiperazine in THF gave the corresponding phosphoramides **20** and **21**, respectively (Scheme 6).



Scheme 6 *Reagents:* i, $POCl_3$, Et_3N, pyridine, Et_2O; ii, H_2O–dioxane, Na_2HPO_4 and KH_2PO_4; iii, Et_2NH; iv, HNRR′

In conclusion, phosphorylation follows the pattern of steric availability of the OH groups. In forskolin it is the 1-position which is phosphorylated first and generally through anchimeric assistance of 9-OH cyclic phosphates are formed; however, in deacetylforskolin **13** 7-OH is phosphorylated first. We have successfully synthesized the 1,9- and 6,7-cyclic phosphates and the 1- and 7-phosphates of forskolin and 7-deacetylforskolin. Some of the water-soluble compounds showed positive inotropic activity in guinea pig atria and others have intraocular-pressure-lowering properties and a few compounds have good blood-pressure-lowering properties. Details of biological activity will be reported elsewhere.

Experimental

^1H NMR spectra were recorded for solutions in $CDCl_3$ unless otherwise stated, with $SiMe_4$ internal standard, on Varian T-60 and JEOL-Fx-90 Q spectrometers. *J*-Values are given in Hz. IR spectra were recorded as KBr disks on Perkin-Elmer 157 and Perkin-Elmer 782 spectrometers. M.p.s were determined on a Kofler hot-stage melting point apparatus and are uncorrected. Light petroleum refers to the fraction boiling in the range 60–80 °C.

7-β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*ene*-1α, 9α-*diyl Cyclophospho*-α/β-*chloridate* **2** *and* **3**.—Forskolin **1** (6.15 g, 15 mmol) was dissolved in a mixture of diethyl ether (50 cm^3), Et_3N (2.1 cm^3, 15 mmol) and pyridine (5 cm^3) and the solution was chilled to −10 °C. A solution of $POCl_3$ (1.38 cm^3, 15 mmol) in diethyl ether (10 cm^3) was added dropwise for 15 min to the chilled solution. The mixture was stirred for 1 h at −10 °C. The temperature was then allowed to rise to ambient and the mixture was stirred for 60 h. Solvent was removed under reduced pressure and the residue was taken up in EtOAc (300 cm^3). The EtOAc layer was washed successively with cold, 1 mol dm^{-3} HCl and water. It was then dried over anhydrous Na_2SO_4 and the solvent was removed. The residue was purified by flash chromatography with 15% MeCN in C_6H_6, to give *compound* **2** (2.45 g, 33%), m.p. 193–195 °C (EtOAc–light petroleum); ν_{max}/cm^{-1} 3704, 3030, 1786, 1747, 1385 and 1136; δ_H 1.08 (3 H, s, Me), 1.3 (3 H, s, Me), 1.38 (3 H, s, Me), 1.61 (3 H, s, Me), 1.73 (3 H, s, Me), 1.88 (1 H, d, *J* 2.9, 5-H), 2.18 (3 H, s, Ac), 2.57 (1 H, d, J_{gem} 18, 12β-H), 3.5 (1 H, d, J_{gem} 18, 12α-H), 4.48 (1 H, t, *J* 2.9, 6-H), 4.99 (1 H, dd, J_{cis} 10.8, J_{gem} 1.98, 15 H, *Z*), 5.20 (1 H, br, 1 H), 5.29 (1 H, d, *J* 3.6, 7-H), 5.29 (1 H, dd, J_{trans} 18, J_{gem} 1.98, 15 H, *E*) and 5.87 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 54.25; H, 6.5; Cl, 7.8; P, 6.1%. $C_{22}H_{32}ClO_8P$ requires C, 53.82; H, 6.57; Cl, 7.22; P, 6.31%), and *compound* **3**, (0.9 g, 12%), m.p. 205 °C (decomp.) (from hot EtOAc), ν_{max}/cm^{-1} 3509, 2941, 1730, 1718, 1299 and 1124; δ_H 1.09 (3 H, s, Me), 1.3 (3 H, s, Me), 1.34 (3 H, s, Me), 1.56 (3 H, s, Me), 1.78 (3 H, s, Me), 1.93 (1 H, d, *J* 2.9, 5-H), 2.15 (3 H, s, Ac), 2.74 (1 H, d, J_{gem} 18.4, 12β-H), 3.16 (1 H, d, J_{gem} 18.4, 12α-H), 4.49 (1 H, t, *J* 2.9, 6-H), 5.08 (1 H, dd, J_{cis} 10.8, J_{gem} 1.86, 15-H *Z*), 5.19 (1 H, br, 1-H), 5.3 (1 H, dd, J_{trans} 18, J_{gem} 1.86, 15-H *E*), 5.32 (1 H, d, *J* 3.6, 7-H) and 5.89 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 53.8; H, 6.6; Cl, 7.6; P, 6.1%).

7β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*ene*-1,9-*diyl* p-(*Methoxyphenyl*)-*Phosphate* **4** *and* **5**.—Forskolin **1** (3.28 g, 8 mmol) was dissolved in pyridine (30 cm^3) and the solution was chilled to −10 °C. Bis-(*p*-methoxyphenyl)phosphorochloridate (4.37 g, 16 mmol) was added dropwise to the stirred solution. The reaction mixture was then gradually allowed to attain room temperature. The mixture was stirred overnight, taken up in EtOAc and washed successively with cold, 1 mol dm^{-3} HCl and water. The EtOAc layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography with 50% EtOAc–light petroleum; compound **6** was the first to elute, followed by α-isomer **4** and β-isomer **5**.

Compound **4** (1.28 g, 28%), m.p. 228 °C (from EtOAc–light petroleum); ν_{max}/cm^{-1} 3419, 2941, 1717, 1493, 1356 and 1190; δ_H 0.51 (3 H, s, Me), 0.99 (1 H, d, *J* 2.7, 5-H), 1.16 (3 H, s, Me), 1.36 (3 H, s, Me), 1.5 (3 H, s, Me), 1.67 (3 H, s, Me), 2.25 (3 H, s, Ac), 2.56 (1 H, d, J_{gem} 18, 12β-H), 3.6 (1 H, d, J_{gem} 18, 12α-H), 3.76 (3 H, s, OMe), 3.96 (1 H, t, *J* 3.6, 6-H), 4.96 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 4.97 (1 H, br, 1-H), 5.21 (1 H, d, *J* 3.6, 7-H), 5.34 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*), 5.93 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H), 6.89 (2 H, d, *J* 9.9, ArH^{3,5}) and 7.19 (2 H, d, *J* 9.9, ArH^{2,6}) (Found: C, 60.1; H, 7.1; P, 5.4. $C_{29}H_{39}O_{10}P$ requires C, 60.20; H, 6.79; P, 5.35%).

Compound **5** (1.31 g, 28%), m.p. 204 °C (EtOAc–light petroleum); ν_{max}/cm^{-1} 3448, 2963, 1717, 1493, 1361, 1237 and 1194; δ_H 1.07 (3 H, s, Me), 1.3 (3 H, s, Me), 1.34 (3 H, s, Me), 1.57 (3 H, s, Me), 1.73 (3 H, s, Me), 1.87 (1 H, s, OH, exchanged with D_2O), 2.06 (1 H, br, 5-H), 2.16 (3 H, s, Ac), 2.57 (1 H, d, J_{gem} 18, 12β-H), 3.19 (1 H, d, J_{gem} 18, 12α-H), 3.76 (3 H, s, OMe), 4.47 (1 H, br t, *J* 3.15, 6-H), 4.91 (1 H, br, 1 H), 4.97 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.27 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H), 5.41 (1 H, d, *J* 3.6, 7-H), 5.84 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H), 6.76 (2 H, d, *J* 9.9, ArH^{3,5}) and 7.06 (2 H, d, *J* 9.9, ArH^{2,6}) (Found: C, 60.0; H, 7.1; P, 5.2%).

Compound **6** (0.05 g, 1.6%) with pyridine as the base and in 13% yield with Hunig's base, m.p. 158–164 °C; ν_{max}/cm^1 3448, 2923, 1706, 1315, 1257, 1068 and 985; δ_H 0.74 (3 H, d, *J* 7.11, 1-Me), 1.2 (6 H, s, 2 × Me), 1.35 (3 H, s, Me), 1.59 (3 H, s, Me), 2.13 (3 H, s, Me), 2.28 (3 H, s, Ac), 2.43 (1 H, d, J_{gem} 13, 12β-H),

3.27 (1 H, d, J_{gem} 13, 12α-H), 4.24 (1 H, dd, $J_{5,6}$ 6.59, $J_{6,7}$ 4.6, 6-H), 4.97 (1 H, dd, J_{cis} 10.56, J_{gem} 1.62, 15-H, *Z*), 5.2 (1 H, dd, J_{trans} 17.26, J_{gem} 1.62, 15-H, *E*), 5.56 (1 H, d, $J_{6,7}$ 4.06, 7-H) and 5.87 (1 H, dd, J_{trans} 17.26, J_{cis} 10.56, 14-H) (Found: C, 67.8; H, 8.6. $C_{22}H_{32}O_6$ requires C, 67.32; H, 8.22%).

7β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*ene*-1α,9α-*diyl* 2-*Hydroxyethyl*-α/β-*phosphate* **7** *and* **8**.—Forskolin **1** (1.23 g, 3 mmol) was dissolved in a mixture of CH_2Cl_2 (20 cm^3) and pyridine (2 cm^3) and the solution was chilled to −10 °C. To this vigorously stirred solution was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (2 cm^3, 14 mmol) dropwise. After 1 h at 0 °C the reaction mixture was kept in the refrigerator (5 °C) for 24 h. It was then diluted with EtOAc and washed successively with cold, mol dm^{-3} HCl and water. The EtOAc layer was dried over anhydrous Na_2SO_4 and the solvent was removed. The residue was purified by flash chromatography with 30% EtOAc–light petroleum (α-isomer **7**) followed by 20% MeCN–$CHCl_3$ (β-isomer **8**).

Compound **7** (0.31 g, 20%), m.p. 198–200 °C (from EtOAc–light petroleum); ν_{max}/cm^{-1} 3389, 2919, 1709, 1356, 1250, 1169 and 1090; δ_H 1.07 (3 H, s, Me), 1.30 (3 H, s, Me), 1.36 (3 H, s, Me), 1.59 (3 H, s, Me), 1.70 (3 H, s, Me), 1.93 (1 H, d, *J* 2.7, 5-H), 2.17 (3 H, s, Ac), 2.54 (1 H, d, J_{gem} 18, 12β-H), 3.51 (1 H, d, J_{gem} 18, 12α-H), 3.7 (2 H, t, *J* 5.4, CH_2CH_2OH), 4.29 (2 H, dd, *J* 10.8 and 5.4, OCH_2CH_2OH), 4.46 (1 H, t, *J* 3.15, 6-H), 4.91 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.01 (merged, 1 H, 1-H), 5.27 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*), 5.34 (1 H, br, 7-H) and 5.86 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 54.3; H, 7.4; P, 5.3. $C_{24}H_{37}O_{10}P{\cdot}H_2O$ requires C, 53.93; H, 7.35; P, 5.60%).

Compound **8** (0.405 g, 26%), m.p. 182 °C (from EtOAc–light petroleum); ν_{max}/cm^{-1} 3401, 2941, 1709, 1351, 1285, 1273, 1235, 1116 and 1057; δ_H 1.09 (3 H, s, Me), 1.3 (3 H, s, Me), 1.36 (3 H, s, Me), 1.55 (3 H, s, Me), 1.7 (3 H, s, Me), 1.97 (1 H, d, *J* 2.7, 5-H), 2.16 (3 H, s, Ac), 2.6 (1 H, d, J_{gem} 18, 12β-H), 3.2 (1 H, d, J_{gem} 18, 12α-H), 3.61 (2 H, t, *J* 5.4, CH_2CH_2OH), 4.2 (2 H, m, CH_2CH_2OH), 4.47 (1 H, t, *J* 3.6, 6-H) 4.94 (1 H, br, 1-H), 4.97 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.29 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*) 5.34 (1 H, br, 7-H) and 5.89 (1-H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 55.4; H, 7.3; P, 5.9%).

7β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*ene*-1α,9α-*diyl Cyclo*-α/β-*phosphite* **9** *and* **10**.—Forskolin **1** (0.82 g, 2 mmol) was dissolved in dry pyridine (10 cm^3) and to the vigorously stirred solution was added bis(trifluoroethyl) hydrogen phosphite (0.91 g, 6 mmol) under nitrogen. The mixture was stirred for 16 h at room temperature, taken up in EtOAc and washed successively with cold, 1 mol aq. dm^{-3} HCl and water. The ethyl acetate layer was dried over anhydrous Na_2SO_4 and the solvent was removed. The solid residue was purified by flash chromatography with 30% EtOAc–light petroleum. Both α-isomer **9** and β-isomer **10** were obtained pure.

Compound **9** (0.36 g, 39%), m.p. 225–227 °C (from EtOAc–light petroleum); ν_{max}/cm^{-1} 3419, 2899, 1730, 1709, 1351, 1253, 1230, 1166, 1114 and 1087; δ_H 1.06 (3 H, s, Me), 1.36 (3 H, s, Me), 1.61 (6 H, s, 2 × Me), 1.71 (3 H, s, Me), 1.94 (1 H, OH, exchangeable), 2.17 (3 H, s, Ac), 2.52 (1 H, d, J_{gem} 18, 12β-H), 3.62 (1 H, d, J_{gem} 18, 12α-H), 4.49 (1 H, t, *J* 2.9, 6-H), 4.97 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.24 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H), 5.11 and 5.34 (2 H, br hump, 1- and 7-H) and 5.91 (1 H, dd J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 58.1; H, 7.8; P, 6.4. $C_{22}H_{33}O_8P$ requires C, 57.89; H, 7.29; P, 6.79%).

Compound **10** (0.12 g, 13%), m.p. 212 °C (from EtOAc–light petroleum); ν_{max}/cm^{-1} 3333, 2877, 1702, 1356, 1262, 1236, 1169 and 1090; δ_H 1.1 (3 H, s, Me), 1.30 (3 H, s, Me), 1.36 (3 H, s, Me), 1.50 (3 H, s, Me), 1.79 (3 H, s, Me), 1.96 (1 H, s, exchangeable, OH), 2.11 (1 H, d, *J* 3.2, 5-H), 2.19 (3 H, s, Ac), 2.77 (1 H, d, J_{gem} 18, 12β-H), 3.01 (1 H, d, J_{gem} 18, 12α-H), 4.51 (1 H, t, *J* 3.2, 6-H), 5.03 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.19 (1 H, br, 1-H), 5.29 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H *E*), 5.38 (1 H, d, *J* 3.6, 7-H) and 5.93 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14 H) (Found: C, 58,2; H, 7.75; P, 6.5%).

6β-*Acetoxy*-8-13-*epoxy*-7β,9α-*dihydroxy*-11-*oxolabd*-14-*en*-1α-*yl Dihydrogen Phosphite Diethylamine Salt* **11**.—Forskolin **1** (0.82 g, 2 mmol) was dissolved in dry benzene (10 cm^3), the solution was chilled to 0 °C, and bis(trifluoroethyl) hydrogen phosphite (1.0 cm^3) was added dropwise to the vigorously stirred mixture at 0 °C. After 2 h at 0 °C the reaction mixture was brought to room temperature and kept for 18 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 25% EtOAc–light petroleum, resulting in the intermediate phosphite, (0.44 g), which is very unstable and immediately reacted further. It (0.23 g, 0.41 mmol) was dissolved in pyridine–water (9:1 v/v, 7 cm^3) and zinc dust (0.2 g) was added to the vigorously stirred mixture. After 30 min the reaction mixture was diluted with EtOAc, filtered and treated with 1 mol dm^{-3} aq. $NaHCO_3$ (EtOAc layer was discarded). The aqueous layer was acidified with cold, dil. HCl and then extracted with EtOAc. The ethyl acetate layer was washed with brine, dried over anhydrous Na_2SO_4 and the solvent was removed. The residue was taken up in MeOH–EtOAc and a slight excess of diethylamine was added; the precipitate was filtered off and washed successively with hot EtOAc and dry diethyl ether. Yield of *compound* **11** was 0.095 g (42%), m.p. 180 °C and then 192–194 °C; ν_{max}/cm^{-1} 3413, 2985, 2703, 1732, 1698, 1441, 1379, 1227, 1198 and 1053; $\delta_H(D_2O)$ 0.96 (3 H, s, Me), 1.03 (3 H, s, Me), 1.26 [6 H, t, *J* 7.2, $N(CH_2Me)_2$], 1.4 (3 H, s, Me), 1.43 (3 H, s, Me), 1.64 (3 H, s, Me), 2.17 (3 H, s, Ac), 2.27 (1 H, d, *J* 2.7, 5-H), 2.58 (1 H, d, J_{gem} 18, 12β-H), 3.04 [4 H, quartet, *J* 7.2, $N(CH_2Me)_2$], 3.38 (1 H, d, J_{gem} 18, 12α-H), 4.37–4.57 (2 H, m, 1- and 7-H), 5.0 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.23 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*), 5.83 (1 H, t, *J* 3.6, 6-H) and 6.13 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 56.9; H, 8.4; N, 2.7. $C_{26}H_{46}NO_9P$ requires C, 57.02; H, 8.47; N, 2.56%).

7β-(tert-*Butyldimethylsiloxy*)-8,13-*epoxy*-6β,9α-*dihydroxy*-11-*oxolabd*-14-*en*-1α-*yl Diphenylphosphate* **14**.—8,13-Epoxy-1α,6β,7β,9α-tetrahydroxylabd-14-en-11-one **13** (3.68 g, 10 mmol) and imidazole (1.7 g, 25 mmol) were dissolved in DMF (80 cm^3) and to this was added TBDMSCl (1.96 g, 13 mmol). The mixture was heated at 35 °C for 2 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 10% EtOAc–C_6H_6 to give an *intermediate* (3 g, 64%), m.p. 166–168 °C; ν_{max}/cm^{-1} 3003, 1702, 1282, 1219, 1117, 1041 and 923; δ_H 0.13 (3 H, s, SiMe), 0.17 (3 H, s, SiMe), 0.91 (9 H, s, Bu^t), 1.05 (3 H, s, Me), 1.26 (3 H, s, Me), 1.38 (3 H, s, Me), 1.39 (3 H, s, Me), 1.56 (3 H, s, Me), 2.46 (1 H, d, J_{gem} 17, 12β-H), 3.16 (1 H, s, J_{gem} 17, 12α-H), 4.04 (1 H, d, *J* 4.53, 7-H), 4.2 (1 H, dd, *J* 2.58 and 4.53, 6-H), 4.56 (1 H, br, 1-H), 4.95 (1 H, dd, J_{cis} 11.1, J_{gem} 1.54, 15-H, *Z*), 5.15 (1 H, dd, J_{trans} 17.48, J_{gem} 1.54, 15-H, *E*) and 6.05 (1 H, dd, J_{trans} 17.48, J_{cis} 11.1, 14-H) (Found: C, 64.9; H, 9.2. $C_{25}H_{46}O_6Si$ requires C, 64.73; H, 9.54%).

This compound (0.484 g, 1 mmol) was added to NaH (0.096 g, 4 mmol) suspended in THF (20 cm^3) and the mixture was stirred for 15 min at room temperature. To this mixture at 0 °C was added diphenyl phosphorochloridate 0.268 g, 1 mmol) dropwise. The reaction mixture was brought to room temperature and was stirred overnight. The solvent was removed and the residue was taken up in EtOAc, washed successively with cold, dil. HCl and water and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography with 4% EtOAc–C_6H_6 to give *compound* **14** (0.348 g, 49%) as a

semisolid, ν_{max}/cm^{-1} 3636, 3003, 1724, 1597, 1493, 1316, 1192 and 1164; δ_H 0.16 (3 H, s, SiMe), 0.17 (3 H, s, SiMe), 0.93 (9 H, s, Bu^t), 1.09 (3 H, s, Me), 1.31 (3 H, s, Me), 1.41 (3 H, s, Me), 1.56 (3 H, s, Me), 1.61 (3 H, s, Me), 1.97 (1 H, d, *J* 2.7, 5-H), 2.65 (1 H, d, J_{gem} 18, 12β-H), 3.26 (1 H, d, J_{gem} 18, 12α-H), 4.11 (1 H, d, *J* 3.6, 7-H), 4.27 (1 H, t, *J* 2.7, 6-H), 5.01 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.01 (1 H, br, 1-H), 5.24 (1 H, dd, J_{trans} 17.1, J_{gem} 1.8, 15-H, *E*), 6.01 (1 H, dd, J_{trans} 17.1, J_{cis} 10.8, 14-H) and 7.19–7.36 (10 H, m, Ph) (Found: C, 63.8; H, 7.7; P, 3.9. $C_{38}H_{55}O_9PSi$ requires C, 63.84; H, 7.76; P, 4.33%).

8,13-*Epoxy*-1α,6β,9α-*trihydroxy*-11-*oxolabd*-14-*en*-7β-*yl Bis*-(2,2,2-*trichloroethyl*)*phosphate* **15**.—A solution of compound **13** (0.736 g, 2 mmol) in pyridine (15 cm^3) was chilled to −10 °C. Bis-(2,2,2-trichloroethyl) phosphorochloridate (0.91 g, 2.4 mmol) was added to the above vigorously stirred mixture, which was stirred for a further 4 h at −10 °C before being taken up in EtOAc and washed successively with cold, 1 mol dm^{-3} HCl and water. The EtOAc layer was dried over anhydrous Na_2SO_4 and the solvent was removed. The oily residue was purified by flash chromatography with 20% EtOAc–light petroleum to give *compound* **15** (0.67 g, 47%), m.p. 155–157 °C (from EtOAc–light petroleum); ν_{max}/cm^{-1} 3448, 3200, 1709, 1266, 1170 and 1096; δ_H 1.04 (3 H, s, Me), 1.26 (3 H, s, Me), 1.41 (6 H, s, 2 × Me), 1.7 (3 H, s, Me), 2.14 (1 H, d, *J* 2.88, 5-H), 2.46 (1 H, d, J_{gem} 17.1, 12β-H), 3.24 (1 H, d, J_{gem} 17.1, 12α-H), 4.64–4.71 (6 H, two broad peaks, 2 × CH_2CCl_3, 1- and 6-H), 4.97 (1 H, dd, J_{cis} 10.8, J_{gem} 1.7, 15-H, *Z*), 5.03 (1 H, br, 7-H), 5.24 (1 H, dd, J_{trans} 18, J_{gem} 1.7, 15-H, *E*) and 6.14 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 40.6; H, 5.35; P, 3.95; Cl, 29.1. $C_{24}H_{35}Cl_6O_9P$ requires C, 40.53; H, 4.96; P, 4.36; Cl, 29.91%).

8,13-*Epoxy*-1α,9α-*dihydroxy*-11-*oxolabd*-14-*ene*-6β,7β-*diyl* 2-*Hydroxyethylcyclic Phosphate* **16**.—To a vigorously stirred, chilled (−5 to 0 °C) solution of compound **13** (3.68 g, 10 mmol) in pyridine (50 cm^3) was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (2.86 cm^3, 20 mmol) dropwise. After 1 h at 0 °C the mixture was kept in a refrigerator (5 °C) for 12 h. It was then taken up in EtOAc (150 cm^3) and washed successively with cold, 1 mol dm^{-3} HCl and water. It was dried over anhydrous Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography with 5% MeCN in $CHCl_3$ to give *compound* **16** (0.71 g, 15%), m.p. 194–195 °C (EtOAc–light petroleum; ν_{max}/cm^{-1} 3077, 2941, 1695, 1449, 1379, 1266, 1190, 1146 and 1086; δ_H 1.10 (3 H, s, Me), 1.19 (3 H, s, Me), 1.35 (3 H, s, Me), 1.41 (3 H, s, Me), 1.65 (3 H, s, Me), 2.34 (1 H, d, J_{gem} 16.2, 12β-H), 2.44 (1 H, br, 5-H), 3.37 (1 H, d, J_{gem} 16.2, 12α-H), 3.70 (2 H, t, *J* 5.4, CH_2CH_2OH), 3.36 (2 H, quintet, *J* 10.8 and 5.4, CH_2CH_2OH), 4.57 (1 H, br, 1-H) 4.8 (1 H, d, *J* 5.7, 7-H), 4.97 (1 H, dd, J_{cis} 10.8, J_{gem} 1.7, 15-H, *Z*), 5.07–5.14 (1 H, br, 6-H), 5.16 (1 H, dd, J_{trans} 17.1, J_{gem} 1.7, 15 H, *E*) and 6.4 (1 H, dd, J_{trans} 17.1, J_{cis} 10.8, 14-H) (Found: C, 54.1; H, 7.7; P, 6.4. $C_{22}H_{35}O_9P{\cdot}H_2O$ requires C, 53.65; H, 7.57; P, 6.29%).

7β-*Acetoxy*-8,13-*epoxy*-6β,9α-*dihydroxy*-11-*oxolabd*-14-*en*-1α-*yl Dihydrogen Phosphate* **17**.—Forskolin **1** (5.5 g, 13.4 mmol) was dissolved in a mixture of CH_2Cl_2 (150 cm^3) and ethyldiisopropylamine (10 cm^3). To this vigorously stirred solution was added 1,2-dibromo-2-phenylethylphosphonic acid (4.0 g, 11.6 mmol). The clear solution was kept at room temperature for 48 h. The solvent was then removed under reduced pressure and the residue was partitioned between EtOAc and 1 mol dm^{-3} aq. $NaHCO_3$ (20 cm^3). The aqueous layer was washed with EtOAc (2 × 5 cm^3), and the combined EtOAc portion was discarded. The aqueous layer was acidified with cold, 1 mol dm^{-3} HCl to pH 2 and was then saturated with NaCl and extracted with EtOAc (5 × 15 cm^3). The EtOAc layer was washed with brine, then dried over anhydrous Na_2SO_4 and the solvent was removed. The residue was crystallized from EtOAc–light petroleum to give *compound* **17** (2 g, 30%), m.p. 149–151 °C; ν_{max}/cm^{-1} 3571, 2958, 1724, 1369, 1257 and 1113; $\delta_H(CDCl_3 + CD_3OD)$ 1.04 (3 H, s, Me), 1.24 (3 H, s, Me), 1.33 (3 H, s, Me), 1.53 (3 H, s, Me), 1.66 (3 H, s, Me), 2.15 (3 H, s, Ac), 2.2 (1 H, d, *J* 3.6, 5 H), 2.27 (1 H, d, J_{gem} 15.12, 12β-H), 3.26 (1 H, d, J_{gem} 15.12, 12α-H), 4.46 (1 H, t, *J* 3.6, 6-H), 4.9 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.04 (1 H, br s, 1-H), 5.19 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*), 5.33 (1 H, d, *J* 3.6, 7-H) and 5.94 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 53.5; H, 7.4; P, 6.4. $C_{22}H_{35}O_{10}P$ requires C, 53.87; H, 7.19; P, 6.32%).

8,13-*Epoxy*-1α,6β,9α-*trihydroxy*-11-*oxolabd*-14-*en*-7β-*yl Dihydrogen Phosphate* **18**.—To a solution of compound **13** (3.68 g, 10 mmol) in a mixture of CH_2Cl_2 (50 cm^3) and ethyldiisopropylamine (5 cm^3) was added 1,2-dibromo-2-phenylethylphosphonic acid (3.44 g, 10 mmol). The clear solution was kept at room temperature for 48 h. The solvent was removed under reduced pressure and the solid residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (2 × 10 cm^3), the EtOAc portion was discarded and the aqueous layer was acidified with cold, 1 mol dm^{-3} HCl to pH 2, and then extracted with EtOAc (3 × 15 cm^3). The EtOAc layer was washed with brine, then dried over anhydrous Na_2SO_4 and the solvent was removed. The solid obtained was crystallized from EtOAc–light petroleum to give *compound* **18** (1.3 g, 29%), m.p. 149 °C (decomp.), 185–189 °C; ν_{max}/cm^{-1} 3371, 2941, 1709, 1449, 1379, 1250 and 1169; $\delta_H(CDCl_3 + CD_3OD)$ 1.06 (3 H, s, Me), 1.29 (3 H, s, Me), 1.44 (3 H, s, Me), 1.46 (3 H, s, Me), 1.76 (3 H, s, Me), 2.11 (1 H, d, *J* 2.88, 5-H), 2.47 (1 H, d, J_{gem} 17.1, 12β-H), 3.29 (1 H, d, J_{gem} 17.1, 12α-H), 4.51–4.79 (3 H, m, 1-, 6- and 7-H), 5.01 (1 H, dd, J_{cis} 10.8, J_{gem} 1.7, 15-H, *Z*), 5.23 (1 H, dd, J_{trans} 18, J_{gem} 1.7, 15-H, *E*) and 6.19 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14 H) (Found: C, 53.1; H, 7.6; P, 7.0. $C_{20}H_{33}O_9P$ requires C, 53.56; H, 7.42; P, 6.91%).

7β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*ene*-1α,-9α-*diyl Cyclophosphate Diethylamine Salt* **19**.—Compound **2** (0.47 g, 0.96 mmol) was dissolved in 1,4-dioxane (5 cm^3)–water (5 cm^3) and Na_2HPO_4 (0.2 g) and KH_2PO_4 (0.05 g) were added. The mixture was refluxed for 2 h on a steam-bath. The solvent was removed under reduced pressure. The residue was diluted with water and made alkaline by addition of saturated aq. $NaHCO_3$. The aqueous layer was extracted with diethyl ether (2 × 15 cm^3) and the clear aqueous layer was acidified with cold, 1 mol dm^{-3} HCl. The separated oil was extracted with EtOAc (4 × 5 cm^3). The EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, and the solvent was removed, under reduced pressure, below 40 °C. The residue was dried in a vacuum desiccator over KOH overnight. It was then dissolved in diethyl ether and freshly distilled diethylamine was added. The precipitate thus formed was filtered off, washed with dry diethyl ether and recrystallized from MeOH–Et_2O to give *compound* **19** (0.4 g, 76%), m.p. 228–230 °C; ν_{max}/cm^{-1} 3419, 2963, 2941, 1714, 1366, 1231, 1176, 1163 and 1099; δ_H 1.01 (3 H, s, Me), 1.29 (12 H, br, 2 × Me + 2 NCH_2Me), 1.55 (3 H, s, Me), 1.67 (3 H, s, Me), 2.10 (1 H merged with Ac, 5-H), 2.14 (3 H, s, Ac), 2.32 (1 H, d, J_{gem} 17.1, 12β-H), 2.83 (4 H, quartet, *J* 7.2, $MeCH_2N$), 3.71 (1 H, d, J_{gem} 17.1, 12α-H), 4.43 (1 H, t, *J* 2.88, 6-H), 4.54 (1 H, br, 1-H), 4.84 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.19 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*), 5.4 (1 H, d, *J* 3.6, 7-H), 5.91 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 56.9; H, 8.1; N, 2.7; P, 5.4. $C_{24}H_{44}NO_9P$ requires C, 57.23; H, 8.13; N, 2.57; P, 5.68%).

7β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*ene*-1α,9α-*diyl* N-[3-(*Dimethylamino*)*propyl*]*cyclophosphoramide Hydrochloride* **20**.—Compound **3** (0.6 g, 1.22 mmol) was dis-

solved in THF (6 cm^3) and 3-(dimethylamino)propylamine (4 cm^3) was added. The clear solution was kept at room temperature for 2 h. The reaction mixture was diluted with EtOAc and washed with water until neutral. The EtOAc layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residual oil was purified by flash chromatography over silica gel with $CHCl_3$ and then 2–10% MeOH–$CHCl_3$ as eluent. The pure material was taken up in dry MeOH and HCl–diethyl ether was added to form the hydrochloride. The precipitate formed was filtered off, washed with dry diethyl ether and crystallized from acetone–diethyl ether to give *compound* **20** (0.29 g, 40%), m.p. 217–218 °C; ν_{max}/cm^{-1} 3419, 2941, 2685, 1717, 1262, 1242 and 1117; δ_H($CDCl_3$ + CD_3OD) 1.03 (3 H, s, Me), 1.29 (6 H, s, 2 × Me), 1.71 (3 H, s, Me), 1.86 (3 H, s, Me), 2.15 (3 H, s, Ac), 2.2–3.4 (14 H, m, 12-H_2 + $CH_2CH_2CH_2NMe_2$) 4.44 (1 H, t, *J* 3.24, 6-H), 4.61 (1 H, br, 1-H), 4.96 (1 H, dd, J_{cis} 10.13, J_{gem} 2, 15-H, *Z*), 5.09 1 H, merged, 7-H), 5.19 (1 H, dd, J_{trans} 17.2, J_{gem} 2.0, 15-H, *E*) and 5.87 (1 H, dd, J_{trans} 17.2, J_{cis} 10.13, 14-H) (Found: C, 53.1; H, 8.0; N, 4.5; Cl, 5.6. $C_{27}H_{46}ClN_2O_8P·H_2O$ requires C, 53.06; H, 7.75; N, 4.59; Cl, 5.80%).

7β-*Acetoxy*-8,13-*epoxy*-6β-*hydroxy*-11-*oxolabd*-14-*enc*-1α, 9α-*diyl* N-(4-*methylpiperazino*)*cyclophosphoramide Hydrochloride* **21**.—Compound **3** (1.0 g, 2.04 mmol) was dissolved in *N*-methylpiperazine (10 cm^3) and the solution was kept at room temperature for 48 h before being diluted with EtOAc and washed with water until neutral. The solution was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography with 7.5% MeOH–$CHCl_3$ and the product was dissolved in EtOAc and HCl in diethyl ether was added. The precipitate thus formed was filtered off, and washed successively with dry EtOAc and diethyl ether to afford *compound* **21** (0.46 g, 38%), m.p. 210–212 °C; ν_{max}/cm^{-1} 3389, 2898, 1705, 1447, 1363, 1242, 1190, 1180, 1095 and 1053; δ_H 1.09 (3 H, s, Me), 1.24 (3 H, s, Me), 1.33 (3 H, s, Me), 1.51 (3 H, s, Me), 1.69 (3 H, s, Me), 2.13 (3 H, s, Ac), 2.25–3.16 (13 H, m, 2 × OH, 5-H, 12-H_2, 4 × CH_2), 4.51 (1 H, t, *J* 3.13, 6-H), 4.97 (1 H, dd, J_{cis} 10.8, J_{gem} 1.8, 15-H, *Z*), 5.03 (1 H, br, 1-H), 5.36 (1 H, dd, J_{trans} 18, J_{gem} 1.8, 15-H, *E*), 5.39 (1 H, d, *J* 3.6, 7-H), 5.83 (1 H, dd, J_{trans} 18, J_{cis} 10.8, 14-H) (Found: C, 52.0; H, 7.5; N, 4.2; Cl, 6.05. $C_{27}H_{44}ClN_2O_8P·2H_2O$ requires C, 51.71; H, 7.71; N, 4.47; Cl, 5.65%).

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