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Potential Protein Kinase C Inhibitors. 8,9,10,11α-Tetrahydro-7αH-7,11-methano-12,12-dimethylcycloocta[de]naphthyl-9-amines

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Abstract

The synthesis of the 9α- and 9β-epimers of 8, 9, 10, 11α-tetrahydro-7αH-7, 11-methano-12, 12-dimethylcycloocta [de] naphthyl-9-amine is described.

Protein kinase C (PKC) is of pivotal importance in certain signal transduction pathways (Borner & Fabbro 1992). Inhibitors of this enzyme may be of use in a wide variety of diseases, including cancer (Borner & Fabbro 1992), asthma (Garland 1989), AIDS (Jakobovits et al 1990), hypertension (Murakawa et al 1988) and rheumatoid arthritis (Hashimoto et al 1992), as well as providing specific modulators of PKC activity for use in the elucidation of signal tranduction pathways and modes of cellular regulation. The microbial metabolite staurosporine **1** is a potent inhibitor (Tamoiki et al 1986) of the enzyme. Analogues have been described (Murray & Warrington 1990; Bit et al 1993; Hill 1994) in an attempt to improve inhibitory selectivity and here we have focussed on a new type of structure **2** based on the planar hetero-aromatic and pyranose ring structures present in **1**. A comparison of the structures of staurosporine **1** and **2** and their superimposition is shown in Fig. 1.

Methods

^{1}H NMR spectra were recorded at 90 MHz on a Perkin-Elmer R32 instrument or at 360 MHz on a Bruker WM360 instrument. Unless otherwise stated solutions were in $CDCl_3$ and chemical shift values are quoted on the d-scale relative to internal tetramethylsilane. ^{13}C NMR were recorded at 22·5 MHz on a Jeol FX90-Q instrument or at 90 MHz on the WM 360. Spectra were measured with broad-band ^{1}H-decoupling (BB) and by the DEPT method where indicated to determine attached protons. Nuclear Overhauser enhancements (NOE) were measured by the difference method using standard Bruker software. A relaxation delay of 10 s followed by a low-intensity pre-saturation pulse of 3 s was applied before each acquisition pulse. A sequence of 8 acquisitions with irradiation at each selected position followed by 8 acquisitions irradiated at a nearby blank position was repeated 24 times. The summed irradiated and blank free-induction decay patterns were subtracted and the result transformed after a line-broadening of 1 Hz. Quantitative values for enhancements were obtained from integrals in the difference spectra. Mass spectra were determined by the SERC Mass Spectrometry Centre at Swansea. Elemental analyses were determined at the School of Pharmacy, London. Melting points were determined on an Electrothermal instrument and are uncorrected. IR spectra on KBr discs were recorded on a Perkin-Elmer 681 spectrophotometer.

The crystal structure of staurosporine was retrieved from the Cambridge Crystallographic Database at SERC, Daresbury. Modelling was conducted using the Nemesis molecular modelling package (Oxford Molecular Ltd 1990) run on an Apple Macintosh LCII Computer.

Ethyl (2,2-dichloro-2,3-dihydro-3-hydroxy-1-oxo-phenalene-3-yl)acetate **11**. The Reformatsky reagent (60 mL of 1·5 mol dm^{-3}, 90 mmol) was added in portions over 10 min to a stirred solution of 2,2-dichloro-2,3-dihydrophenalene-1,3-dione (Gudriniece et al 1960) **9** (19·4 g, 73 mmol) in anhydrous tetrahydrofuran (THF, 120 mL) whereupon a fine white solid precipitated. The mixture was stirred for 30 min, filtered and the residue dried in a vacuum oven to give the zinc salt intermediate **10** as a pale yellow solid (34·8 g). The complex was decomposed by addition to a vigorously stirred mixture of water (100 mL), sulphuric acid (18 mol dm^{-3}, 5 mL) and dichloromethane (100 mL). The organic phase was separated, washed with water (2 × 50 mL) and saturated aqueous sodium hydrogen carbonate (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a golden yellow oil (23·4 g) which, on dissolution in hot isopropyl ether and subsequent cooling to room temperature, afforded a crystalline solid which was collected and dried in a low temperature vacuum oven to give the β-hydroxyester **11** (17 g), m.p. 64–65°C. Cooling the filtrate to 0°C furnished a second crop (2·5 g; total yield = 19·5 g, 76%). Recrystallisation of a portion from isopropyl ether afforded an analytical sample as colourless rhombs, m.p. 65–65·5°C (Found: C, 57·82; H, 3·91. $C_{17}H_{14}Cl_2O_4$ requires C, 57·79; H, 4·00%); υ_{max} (nujol)/cm^{-1} 3380 (OH), 1720 (ester C=O), 1710 (ketone C=O) and

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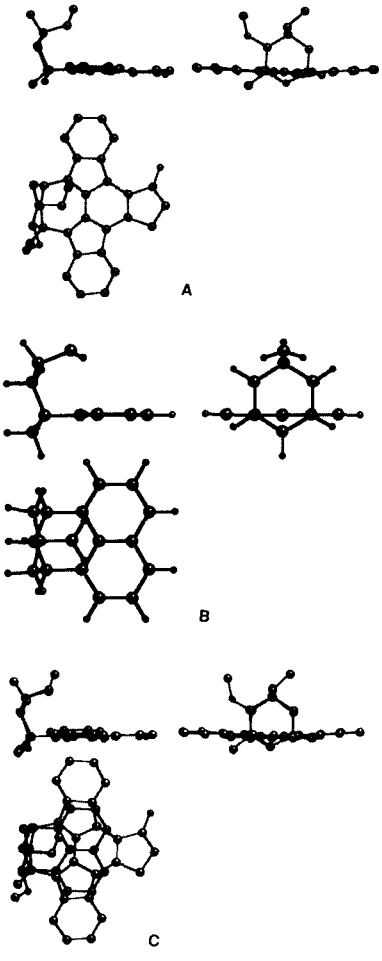


FIG. 1. A. Crystal structure of staurosporine (**1**). B. Graphics model of **2**. C. Superimposition of **1** and **2**. The crystal structure of **1** was retrieved from the Cambridge Crystallographic Database, SERC, Daresbury and **2** was modelled using Nemesis (Oxford Molecular Ltd, 1990). Three views are shown: side, front and top.

1585 (C=C, Ar); δ_H(90 MHz) 8·55–7·43 (6H, m, Ar-*H*), 5·58 (1H, s, O*H*), 3·99 (2H, q, J = 8 Hz, CH_2), 3·16 (1H, d, J_{BA} = 17 Hz, CH_A), 2·68 (1H, d, J_{AB} = 17 Hz, CH_B), 1·01 (3H, t, J = 8 Hz, CH_3).

Ethyl (2,2,3-trichloro-2,3-dihydro-1-oxo-phenalene-3-yl)acetate **13**. Thionyl chloride (11 mL, 151 mmol) was added in one portion to a stirred suspension of the β-hydroxyester zinc salt **10** (29 g, 58 mmol) in anhydrous benzene (100 mL) and the mixture stirred for 20 min at room temperature and then for 70 min at 50°C. The mixture was then cooled to room temperature and decanted from a red gum on the sides of the vessel. Removal of the solvent under reduced pressure afforded a red oil (22 g) which was then heated on a steam bath for 5 min with isopropyl ether (50 mL) and the solution decanted from some further red tar. The solution, on cooling and seeding, rapidly deposited the highly crystalline trichloro ester 13 as pale yellow, fine needles (15 g, 69%), m.p. 88·5–89·5°C. Found: C, 54·93; H, 3·47. $C_{17}H_{13}Cl_3O_3$ requires C, 54·91; H, 3·53%; υ_{max} (nujol)/cm^{-1} 1740 (ester C=O), 1715 (ketone C=O) and 1585 (C=C, Ar); δ_H (90 MHz) 8·57–7·50 (6H, m, Ar-*H*), 3·73 (2H, q, J = 8 Hz, CH_2), 3·45 (1H, d, J_{BA} = 15 Hz, CH_A), 2·90 (1H, d, J_{AB} = 15 Hz, CH_B), 0·87 (3H, t, J = 8 Hz, *Me*).

Ethyl (2,2-dichloro-1-oxo-phenalene-3-ylidene)acetate (**12**). Phosphorus pentoxide (700 mg, 4·9 mmol) and the β-hydroxyester **11** (1·0g, 2·8 mmol) were added in turn to a stirred suspension of Celite (2 g) in dry benzene (20 mL) and the mixture stirred vigorously at 80–90°C for 1 h. The mixture was then filtered, the residue washed with benzene (2 × 10 mL) and the filtrate washed with water (2 × 30 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow/orange oil (800 mg, 85%). Eventually, crystallization from methanol afforded the α,β-unsaturated ester as a mixture of geometric isomers (non-respective ratio of 5 : 3) as small yellow prisms, m.p. 99–103°C. Found: C, 61·01; H, 3·70. $C_{17}H_{12}Cl_2O_3$ requires C, 60·90; H, 3·61%; υ_{max} (nujol)/cm^{-1} 1755 (ester C=O), 1715 (ketone C=O) 1645 (C=C) and 1585 (C=C, Ar); δ_H (90 MHz) 8·88–7·52 (m, Ar-*H*), 7·10 (s, minor isomer, C*H*), 6·57 (s, major, C*H*), 4·30 (q, J = 8 Hz, CH_2), 4·24 (q, J = 8 Hz, CH_2), 1·25 (t, J = 8 Hz, CH_3), 1·19 (t, J = 8 Hz, CH_3).

2,3-Dihydro-2-methylphenalene-1,3-dione (**14a**). A stirred mixture of naphthalic anhydride (113·8 g, 0·574 mol, recrystallized from DMF), diethyl methylmalonate (250 g, 1·435 mol) and zinc chloride (113.8 g, 0.835 mol) was heated under reflux at 145-155°C for 5 h. The reaction mixture was then heated at 170-180°C with vigorous stirring for 1 h during which time most of the ethanol produced during condensation and excess diethyl methylmalonate was removed by distillation, leaving the product as an orange solid. The cooled product was then dissolved in aqueous sodium hydroxide (4 mol dm^{-3}, ca. 1·3 dm^{-3}) and the resulting deep red solution filtered and acidified with excess hydrochloric acid (10 mol dm^{-3}). The precipitated solid was collected, washed with water (6 × 300 mL) and dried in a vacuum oven at 70°C to give the crude methyldione **14a** as a yellow solid (101 g, 84%) which was used without further purification. An analytical sample was obtained by crystallization of a portion from ethanol-water to give **14a** as orange needles, m.p. 180–182°C [Literature (Geissman & Morris 1944) m.p. 183–185°C]; υ_{max} (KBr)/cm^{-1} 3650–2400 br (OH, CH), 1635 (C=C) and 1610 (C=O); δ_H (90 MHz; $(CD_3)_2SO$) 8·49–8·21 (4H, m, 4,6,7,9-*H*), 7·81 (2H, t, J = 8 Hz, 5,8-*H*), 2·12 (3H, s, CH_3).

2,3-Dihydro-2,2-dimethylphenalene-1,3-dione (**15**). (i) Potassium *tert*-butoxide (3·12 g, 25.5 mmol) was added to a stirred slurry of partially dissolved dione **3a** (5·0 g, 25·5 mmol) in anhydrous DMF (25 mL). The red mixture was stirred at room temperature for 5 min after which methyl iodide (5·43 g, 38·3 mmol) was added and the mixture stirred for ca. 20 min until neutral. Further portions of potassium *tert*-butoxide (3·12 g, 25·5 mmol) and methyl iodide (5·43 g, 38·3 mmol) were added and the suspension heated at 70°C for ca. 20 min until homogenous. The mixture was cooled and poured into a vigorously stirred mixture of toluene (100 mL) and water (100 mL). The organic phase was separated, washed with water (3 × 100 mL) and aqueous ammonia (5 mol dm^{-3}, 50 mL), rewashed with water (3 × 100 mL), dried ($MgSO_4$), clarified with decolourising carbon and

concentrated under reduced pressure to give a light brown oil (2·55 g) which, on chromatography over silica with chloroform as eluent, gave the dimethyldione **15** as a white crystalline solid (1·34 g, 23%). Recrystallization of a portion from methanol afforded an analytical sample as needles, m.p. 98–100°C [Literature (Geissman & Morris 1944) m.p. 99–101°C]; υ_{max} (KBr)/cm^{-1} 2980, 2960 (CH), 1700, 1675 (C=O) and 1585 (C=C, Ar); δ_H(90 MHz) 8·44 (2H, d, J = 8 Hz, 4,9-H), 8·23 (2H, d, J = 8 Hz, 6,7-H), 7·75 (2H, t, J = 8 Hz, 5,8-H), 1·55 (6H, s, CH_3).

(ii) Sodium methoxide in absolute methanol (81·6 mL of 30%w/w, 0·428 mol) was added dropwise, with stirring to a refluxing mixture of the methyldione **14a** (90 g, 0·428 mol), methyl iodide (136·6 g, 0·963 mol), and dry acetone (300 mL) over 3 h. The resulting dark red solution was concentrated under reduced pressure and the residual oil treated with aqueous sodium hydroxide (2 mol dm^{-3}, 200 mL). The precipitated solid was collected, washed with water (5 × 150 mL) and dried to give a mixture of the dimethyldione **15** and the C,O-dimethyl enol ether **14c** as a yellow/brown solid (79·4 g, 83%). A portion of the mixture (3·50 g) was purified by column chromatography on silica with chloroform as eluent and afforded the dimethyldione **15** as a white crystalline solid (2·61 g, ca. 74% based on total product yield) followed by **14c** as a bright yellow solid (0·85 g, ca. 24%). Recrystallization of the former from methanol afforded **15** as needles, m.p. 97–99°C (undepressed by admixture with the previous material).

Ethyl (2,3-dihydro-3-hydroxy-2,2-dimethyl-1-oxo-phenalene-3-yl)ethanoate (**17**). (i) The pure dimethyldione **15** (0·5 g, 2·23 mmol) was added, with stirring, under nitrogen to the Reformatsky reagent (6 mL of 1 mol dm^{-3}, 6 mmol) at room temperature whereupon the zinc salt **16** precipitated immediately as a fine white solid. The suspension was stirred for 1 h and then quenched by the addition of hydrochloric acid (1 mol dm^{-3}, 10 mL) and toluene (20 mL). The organic phase was separated, washed with water (2 × 20 mL) and saturated aqueous sodium hydrogen carbonate (20 mL), dried ($MgSO_4$), clarified with decolourising carbon and concentrated under reduced pressure to afford a pale green oil which, on refrigeration, crystallized to furnish the β-hydroxyester **17** as a white solid (0·69 g, 99%), m.p. 58–60°C. A portion was recrystallized from cyclohexane to give **17** as needles, m.p. 64–65°C. Found C, 72·85; H, 6·38; M$^+$, 312·1360. $C_{19}H_{20}O_4$ requires C, 73·04; H, 6·46%; M$^+$, 312·1362); υ_{max} (KBr)/cm^{-1} 3500 (OH), 3060, 2990 (CH), 1725 (C=O ester), 1685 (C=O, ketone) and 1585 (C=C, Ar); δ_H(90 MHz) 8·35–7·43 (6H, m, Ar-*H*), 5·29 (1H, s, O*H*), 3·91 (2H, q, J = 8 Hz, CH_2), 2·87 (1H, d, J_{BA} = 16 Hz, CH_A), 2·42 (1H, d, J_{BA} = 16 Hz, CH_B), 1·47 (3H, s, CH_3), 1·10 (3H, s, CH_3), 0·95 (3H, t, J = 8 Hz, CH_3).

(ii) The Reformatsky reagent (280 mL of 1.5 mol dm^{-3}, 0·421 mol) was added in portions to a solution of the dimethylated product mixture of **15** and **14c** (78·6 g, 0·351 mol) in dry THF (280 mL) at room temperature, whereupon ca. 30 s after completion of the above treatment, the zinc salt **16** precipitated as a fine white solid. The suspension was stirred for 30 min; the precipitate was collected, washed with anhydrous ether (2 × 100 mL) and dried to give the zinc complex as an off-white solid (123·2 g, 0·270 mol). The complex was decomposed by vigorously stirring into a mixture of hydrochloric acid (2 mol dm^{-3}, 200 mL) and dichloromethane (200 mL). The organic phase was separated, washed with water (2 × 100 mL) and saturated aqueous sodium hydrogen carbonate (2 × 100 mL), dried ($MgSO_4$), clarified with decolourising carbon and the solvent removed under reduced pressure to afford a pale orange oil which, on refrigeration, solidified to give the crude product as a pale yellow solid (81·06 g, 74%). Trituration of the product with light petroleum gave the β-hydroxyester **17** as a white solid (70·2 g, 64%) which was used without further purification. Crystallization of a portion from cyclohexane gave needles, m.p. 63–65°C, undepressed by admixture with the previous material.

Ethyl (2,3-dihydro-2,2-dimethyl-1-oxo-phenalene-3-ylidene)-ethanoate (**18**). Celite (50 g) and phosphorus pentoxide (28·39 g, 200 mmol) were added in turn to a stirred solution of the β-hydroxyester **17** (50·0 g, 160 mmol) in dry benzene (250 mL) and the reaction mixture vigorously stirred at 80–90°C for 1·5 h. The mixture was then filtered, the residue washed with ether (2 × 50 mL) and the combined filtrates washed with water (2 × 300 mL), dried ($MgSO_4$), clarified with decolourising carbon and the solvent removed under reduced pressure to afford an orange oil which, on cooling in ice-water, gave the crude product as a pale orange solid (44·53 g). Trituration with light petroleum (ca. 100 mL) gave the α,β-unsaturated ester **18** as an off-white solid (37·16 g, 79%) which was used without further purification. Crystallization of a portion from methanol furnished **18** as white fluffy needles, m.p. 77–78°C. Found: C, 77·68; H, 6·07. $C_{19}H_{18}O_3$ requires C, 77·52; H, 6·17%); υ_{max} (KBr)/cm^{-1} 3030, 2990 (CH), 1720 (C=O, ester), 1685 (C=O, ketone), 1635 (C=C) and 1585 (C=C, Ar); δ_H(90 MHz) 8·38–7·40 (6H, m, Ar-*H*), 6·18 (1H, s, C*H*), 4·18 (2H, q, J = 8 Hz, CH_2), 1·46 (6H, s, CH_3), 1·20 (3H, t, J = 8 Hz, CH_3).

Ethyl (1-carbethoxymethyl-2,3-dihydro-1-hydroxy-2,2-dimethyl-phenalene-3-ylidene)ethanoate (**19**). The Reformatsky reagent (140 mL of 1·5 mol dm^{-3}, 210 mmol) was added to a stirred solution of the α,β-unsaturated ester **19** (41·0 g, 139 mmol) in anhydrous THF (140 mL). The reaction mixture was quenched after 5 min by the addition and vigorous mixing of a solution of hydrochloric acid (2 mol dm^{-3}, 150 mL) and ether (150 mL), TLC ($CHCl_3$) having revealed the complete absence of starting material. The organic layer was separated, washed with water (2 × 100 mL) and saturated aqueous sodium hydrogen carbonate (1 × 100 mL), dried ($MgSO_4$), clarified with decolourising carbon and concentrated under reduced pressure to give an orange oil which on cooling in ice-water furnished the crude product as a pale orange solid (51·53 g, 97%). Trituration with light petroleum (2 × 100 mL) furnished the β-hydroxydiester **19** as a white solid (45·15 g, 85%). Crystallization of a portion from methanol gave an analytical sample as needles, m.p. 109–110°C. Found: C, 72·31; H, 6·87. $C_{23}H_{26}O_5$ requires C, 72·22; H, 6·86%); υ_{max} (KBr)/cm^{-1} 3450 (OH), 3060, 2990 (CH), 1710br (C=O) and 1630 (C=C); δ_H(90 MHz) 8·01–7·35 (6H, m, Ar-*H*), 6·16 (1H, s, C*H*), 5·50 (1H, s, O*H*), 4·33 (2H, q, J = 8 Hz, CH_2), 4·14 (2H, q, J = 8 Hz, CH_2), 2·98 (1H, d, J_{BA} = 16 Hz, CH_A), 2·68 (1H, d, J_{BA} = 16 Hz, CHB),

1·56 (3H, s, CH_3), 1·37 (3H, t, J = 8 Hz, CH_3), 1·18 (3H, t, J = 8 Hz, CH_3), 1·05 (3H, s, CH_3).

Diethyl (2,3-dihydro-2,2-dimethylphenalene-1,3-bisylidene)-diacetate (**20**). (i) Celite (5·0 g) and phosphorus pentoxide (2·32 g, 16·34 mmol) were added in turn to a stirred solution of the β-hydroxydiester **19** (5·0 g, 13·1 mmol) in anhydrous benzene (50 mL) and the mixture stirred vigorously and heated under reflux for 1·5 h. The mixture was then filtered, the residue washed with ether (25 mL) and the combined filtrates washed with water (2 × 50 mL), dried ($MgSO_4$), clarified with decolourising carbon and concentrated under reduced pressure to afford an orange oil (4·62 g). The oil was dissolved in hot methanol and left to stand in a refrigerator overnight after which a white crystalline solid (1·06 g) was isolated. Further concentration and refrigeration of the mother liquors furnished a second crop which was combined with the first to give a total yield of the α,β-unsaturated diester **20** of 1·66 g (35%). Recrystallization of a portion from methanol afforded an analytical sample as needles, m.p. 86–87°C. Found: C, 75·37; H, 6·53; M$^+$, 364·1675. $C_{23}H_{24}O_4$ requires C, 75·79; H, 6·64%; M+, 364·1675); υ_{max} (KBr)/cm^{-1} 3030, 2990 (CH), 1710 (C=O) and 1630 (C=C); δ_H(90 MHz) 7·95–7·27 (6H, m, Ar-*H*), 6·05 (2H, s, C*H*), 4·20 (4H, q, J = 8 Hz, CH_2), 1·35 (6H, s, CH_3), 1·23 (6H, t, J = 8 Hz, CH_3).

(ii) Dry pyridine (14·3 mL, 176 mmol) was added dropwise to a stirred solution of the β-hydroxydiester **19** (22·5 g, 59 mmol) and thionyl chloride (8·58 mL, 118 mmol) in dry benzene (230 mL) immersed in an ice-water bath, whereupon a dense white precipitate formed in the solution. The suspension was stirred for 1·5 h at 2–10°C and then poured into a vigorously stirred mixture of ether (200 mL) and hydrochloric acid (1 mol dm^{-3}, 130 mL). The organic layer was separated, washed with water (2 × 100 mL) and saturated aqueous sodium hydrogen carbonate (2 × 100 mL), dried ($MgSO_4$) and clarified with decolourising carbon. Removal of the solvent under reduced pressure gave the crude product as a pale yellow oil (20·93 g) which crystallized on refrigeration to give a mixture of the α,β-unsaturated diester **20** and the β-chlorodiester **21** which was used without further purification; υ_{max} (KBr)/cm^{-1} 3050, 2990, 2940 (CH), 1720 br (C=O) and 1630 (C=C); δ_H(90 MHz) 8·15–7·26 (m, Ar-*H*), 6·13 (s, C*H*), 6·05 (s, C*H*), 4·19 (q, J = 8 Hz, CH_2), 3·89 (q, J = 8 Hz, CH_2), 3·15 (d, J_{BA} = 15 Hz, CH_A), 2·95 (d, J_{AB} = 15 Hz, CH_B), 1·63 (s, CH_3), 1·34 (s, CH_3), 1·22 (t, J = 8 Hz, CH_3), 1·06 (s, CH_3), 0·92 (t, J = 8 Hz, CH_3).

Diethyl 2,3-dihydro-2,2-dimethylphenalene-1,3-diacetate (**22**). (i) A solution of the α,β-unsaturated diester **20** (1·0 g, 2·75 mmol) in absolute ethanol (20 mL) containing 10% palladium on charcoal (0·2 g) was shaken in hydrogen until no further uptake of gas was observed. The catalyst was removed by filtration through a bed of Celite and the filtrate concentrated under reduced pressure to give a white solid (0·98 g). Crystallization from light petroleum afforded the saturated diester **22** as prisms, m.p. 87–88°C. Found: C, 74·98; H, 7·72. $C_{23}H_{28}O_4$ requires C, 74·96; H, 7·66%.); υ_{max} (KBr)/cm^{-1} 3060, 2980, 2900 (CH), 1730 (C=O) and 1595 (C=C, Ar); δ_H (90 MHz) 7·92–7·33 (6H, m, Ar-*H*), 4·29 (4H, q, J = 8 Hz, CH_2), 3·65 (2H, dd, J_{MA} = 4 Hz, J_{XA} = 10 Hz, H_A), 3·10 (2H, dd, J_{AM} = 4 Hz, J_{XM} = 18 Hz, H_M), 2·62 (2H, dd, J_{AX} = 10 Hz, J_{MX} = 18 Hz, H_X), 1·38 (6H, t, J = 8 Hz, CH_3), 1·27 (3H, s, CH_3), 1·17 (3H, s, CH_3).

(ii) A solution of the crude α,β-unsaturated diester (**22** + **21**) (17·68 g) in absolute ethanol (100 mL) containing 10% palladium on charcoal (8·84 g) was shaken in an atmosphere of hydrogen until no further uptake of gas was observed. The catalyst was removed by filtration through a bed of Celite and the filtrate concentrated under reduced pressure to give a white solid (16·62 g) which was shown by TLC to contain a mixture of components. Successive crystallization and recrystallization from light petroleum with seeding afforded the pure saturated diester **22** as a white crystalline solid (10·1 g, 56%); m.p. 87–88°C, undepressed by admixture with an authentic sample.

Ethyl (8,9,10,11α-tetrahydro-7αH-7,11-methano-12,12-dimethyl-9-oxo-cycloocta-[de]naphthalene)-10-carboxylate (**23**). Potassium *tert*-butoxide (4·01 g, 32·81 mmol) was added in portions to a stirred solution of the the diester **22** (12·0 g, 32·76 mmol) in dry toluene (100 mL) and the mixture heated in an oil bath at 50°C for 30 min. The resulting brown solution was cooled and then quenched by the addition of a mixture of hydrochloric acid (1 mol dm^{-3}, 100 mL) and ether (100 mL). The organic layer was separated, washed with water (2 × 100 mL) and saturated aqueous sodium hydrogen carbonate (2 × 100 mL), dried ($MgSO_4$) and clarified with decolourising carbon. Removal of the solvent under reduced pressure afforded a pale yellow oil (10·46 g) which, on chromatography over silica with toluene as eluent, furnished the β-ketoester **23** (6·64 g, 63%). Crystallization of a portion from ethanol gave an analytical sample as white needles, m.p. 97–98°C. Found: C, 77·85; H, 6·98. $C_{21}H_{22}O_3$ requires C, 78·22; H, 6·88%.); υ_{max} (KBr)/cm^{-1} 3070 w, 3040 w, 2990 s, 2960, 2940, 2910 s, 2870 (CH), 1640 br (C=O, chelated enolic β-ketoester), 1605 (C=C, Ar), 1275, 1055 and 775; δ_H (360 MHz) 12·2 (1H, s, O*H*), 7·67 (1H, d, J = 8 Hz, Ar-*H*), 7·62 (1H, d, J = 8 Hz, Ar-*H*), 7·40 (1H, t, J = 7 Hz, Ar-*H*), 7·37 (1H, d, J = 7 Hz, Ar-*H*), 7·32 (1H, d, J = 7 Hz, Ar-*H*), 7·26 (1H, t, J = 7 Hz, Ar-*H*), 4.32 (1H, dq, J_{BA} = 12.2Hz, J_{XA} = 7·1Hz, CH_A), 4·22 (1H, dq, J_{AB} = 12·2 Hz, J_{XB} = 7·1Hz, CH_B), 3·69 (1H, s, 11-*H*), 3·04 (1H, br s, 7-H_Y), 3·02 (1H, dd, J_{DC} = 21·3 Hz, J_{YC} = 6·1Hz, 8-H_C), 2·42 (1H, dd, J_{CD} = 21·3 Hz, J_{YD} = 5·1 Hz, 8-H_D), 1·44 (3H, t, $J_{A/BX}$ = 7·1 Hz, CH_3), 1·28 (3H, s, CH_3), 0·83 (3H, s, CH_3); δ_C (90 MHz; BB and DEPT) 172·08 (*C*), 171·63 (*C*), 139·99 (*C*), 133·72 (*C*), 127·59 (*C*), 126·61 (*C*H-Ar), 125·91 (*C*H-Ar), 125·82 (*C*H-Ar), 125·43 (*C*H-Ar), 125·33 (*C*H-Ar), 123·98 (*C*H-Ar), 103·31 (*C*), 60·54 ($CO_2CH_2CH_3$), 44·93 (7/11-*C*H), 44·76 (7/11-*C*H), 37·52 (8-CH_2), 33·25 (*C*), 26·93 (CH_3), 25·57 (CH_3), 14·48 ($CO_2CH_2CH_3$).

8,9,10,11α-Tetrahydro-7αH-7,11-methano-12,12- dimethyl-cycloocta[de]naphthalen-9-one (**24**). A mixture of the β-keto ester **23** (16·10 g, 50 mmol), hydrochloric acid (5 mol dm^{-3}, 100 mL) and acetic acid (16·7 mol dm^{-3}, 20 mL) was heated under reflux for 10 h and left stirring overnight at room temperature. A mixture of benzene (50 mL) and ether (50 mL) was then added and, after vigorous shaking, the

organic phase was separated, washed with water (3 × 100 mL) and saturated aqueous sodium hydrogen carbonate (1 × 100 mL), dried ($MgSO_4$), decolourised and concentrated under reduced pressure to give the ketone **24** as a white crystalline solid (12·33 g, 99%). Recrystallization of a portion from ether gave the ketone as needles, m.p. 140–141°C. Found: C, 86·66; H, 7·19. $C_{18}H_{18}O$ requires C, 86·35; H, 7·25%.); υ_{max} (KBr)/cm^{-1} 2980, 2960, 2900 (CH), 1705 (C=O) and 1600 (C=C, Ar); δ_H (360 MHz) 7·65 (2H, d, J = 8 Hz, 3,4-*H*), 7·37 (2H, t, J = 8 Hz, 2,5-H), 7·18 (2H, d, J = 7 Hz, 1,6-*H*), 3·22 (2H, d, J_{BA} = 5 Hz, 7,11-H_A), 3·05 (2H, dd, J_{AB} = 5 Hz, J_{CB} = 16·1 Hz, 8,10-H_B), 2·40 (2H, d, J_{BC} = 16·1 Hz, 8,10-H_C), 1·60 (3H, s, 13-CH_3), 0·93 (3H, s, 14-CH_3).

8,9,10,11α-Tetrahydro-7αH-7,11-methano-12,12-dimethylcycloocta[de]-naphthyl-9β-amine 2S and N,N-di(8,9,10,11α-tetrahydro-7αH-7,11-methano-12,12-dimethylcycloocta[de]naphth-9β-yl)amine (**26**). A mixture of the ketone 24 (1.00 g, 4.00 mmol), formic acid (2·76 g, 60 mmol) and freshly distilled formamide (0·90 g, 20 mmol) was heated under reflux in an oil bath at 165–170°C for 12 h. The cooled reaction mixture was then taken up in ethyl acetate (50 mL), washed with water (4 × 50 mL), dried ($MgSO_4$), clarified with decolourising carbon and concentrated under reduced pressure to give a yellow oil (1·11 g). Purification by chromatography on silica with chloroform as eluent gave firstly the crude formyl derivative of **24** and on further elution with methanol, the secondary amine **26** as a white solid (340 mg). Crystallization of the latter from propan-2-ol furnished the pure amine **26** as colourless plates, m.p. 161–162°C. Found: C, 88·48; H, 8·05; N, 2·95; M^+, 485·3082. $C_{36}H_{39}N$ requires C, 89·02; H, 8·10; N, 2·89%; M^+, 485·3082.); υ_{max} (KBr)/cm^{-1} 3405 (NH), 3060, 3040, 3000, 2940, 2870 (CH), 1600 (C=C, Ar), 1360, 1180, 1120, 830 and 780; δ_H (360 MHz) 7·49 (4H, d, J = 8 Hz, 3,4-*H*), 7·19 (4H, t, J = 8 Hz, 2,5-*H*), 6·83 (4H, d, J = 7 Hz, 1,6-*H*), 2·40 (4H, br s, 7,11-H_A), 2·09 (2H, t, J_{BD} = 5·6 Hz, 9-H_D), 1·69 (4H, dt, J_{AB} = ~4 Hz, J_{CB} = 14·3 Hz, J_{DB} = 5·6 Hz, 8,10-H_B), 1·57 (1H, br s, N*H*), 1·07 (6H, s, 13-CH_3), 0·68 (4H, d, J_{BC} = 14·3 Hz, 8,10-H_C), 0·58 (6H, s, 14-CH_3).

A mixture of the above crude formyl intermediate (580 mg), hydrochloric acid (5 mol dm^{-3}, 10 mL) and acetic acid (16·7 mol dm^{-3}, 2·5 mL) was heated under reflux for 4 h. The mixture was then concentrated under reduced pressure and the residue partially dissolved in water (20 mL). The resulting milky suspension was washed with dichloromethane (2 × 20 mL) and then treated with aqueous sodium hydroxide (1 mol dm^{-3}) until pH 9–10. The alkaline mixture was then extracted with dichloromethane (2 × 20 mL) and the combined extracts washed with water (2 × 20 mL), dried ($MgSO_4$), decolourised and concentrated under reduced pressure to give a pale green oil which, on cooling, crystallized to give the primary amine **25** as a white solid (270 mg, 27%). Recrystallization of a portion from isopropyl ether gave an analytical sample of 25 as needles, m.p. 119–120°C. Found: C, 85·82; H, 8·42; N, 5·66; M^+, 251·1674. $C_{18}H_{21}N$ requires C, 86·00; H, 8·43; N, 5·57%; M^+, 251·1674.); υ_{max} (KBr)/cm^{-1} 3620–3200 br, 3400 (NH), 3060, 3040, 2990, 2960, 2940, 2880 (CH), 1600 (C=C, Ar), 1180, 1100, 830, 800, 790 and 760; δ_H (360 MHz) 7·65 (2H, d, J = 8 Hz, 3,4-*H*), 7·40 (2H, t, J = 8 Hz, 2,5-*H*), 7·30 (2H, d, J = 7 Hz, 1,6-*H*), 3·10 (1H, t, J_{BD} = 6·2 Hz, 9-H_D), 2·85 (2H, br s, 7,11-H_A), 2·57 (2H, dt, J_{AB} = ~3 Hz, J_{CB} = 14·5 Hz, J_{DB} = 6·2 Hz, 8,10-H_B), 1·78 (2H, d, JBC = 14·5 Hz, 8,10-H_C), 1·29 (3H, s, 13-CH_3), 0·77 (3H, s, 14-CH_3), 0·61 (2H, s, NH_2).

8,9,10,11α-Tetrahydro-7αH-7,11-methano-12,12-dimethylcycloocta[de]naphth-9β-ol (**27**). A solution of the ketone 24 (2 g, 8·00 mmol) in dry tetrahydrofuran (20 mL) was added dropwise to a stirred solution of lithium aluminium hydride in tetrahydrofuran (1 mol dm^{-3}, 17·6 mL) under nitrogen. The resulting clear solution was stirred at room temperature for 1 h and then quenched by the sequential addition of water (0·67 mL), aqueous sodium hydroxide (0·67 mL of 15% w/v) and water (2 mL). The mixture was then filtered and the filtrate clarified with decolourising carbon and concentrated under reduced pressure to give the alcohol **26** as a white crystalline solid (1·85 g, 92%). Recrystallization of a portion from ether gave an analytical sample as needles, m.p. 117–119°C. Found: C, 85·43; H, 7·81. $C_{18}H_{20}O$ requires C, 85·66; H, 7·99%.); υ_{max}(KBr)/cm^{-1} 3610 (OH), 3060, 3040, 2940, 2880 (CH) and 1600 (C=C, Ar); δ_H(360 MHz) 7·68 (2H, d, J = 8 Hz, 3,4-*H*), 7·40 (2H, t, J = 8 Hz, 2,5-*H*), 7·30 (2H, d, J = 7 Hz, 1,6-*H*), 3·92 (1H, t, J_{BD} = 5·2 Hz, 9-H_D), 2·87 (2H, t, J_{BA} = ~3 Hz, J_{CA} = ~2 Hz, 7,11-H_A), 2·50 (2H, dt, J_{AB} = ~3 Hz, J_{CB} = 14·7 Hz, J_{DB} = 5·2 Hz, 8,10-H_B), 2·00 (2H, dd, JBC = 14·7 Hz, J_{AC} = ~2 Hz, 8,10-H_C), 1·29 (3H, s, 13-CH_3), 0·76 (3H, s, 14-CH_3).

8,9,10,11α-Tetrahydro-7αH-7,11-methano-12,12-dimethylcycloocta[de]naphthyl-9β-methanesulphonate (**28**). Methanesulphonic anhydride (1.26 g, 7.29 mmol) was added to a stirred solution of the alcohol 27 (1.23 g, 4.88 mmol) in dry dichloromethane (50 mL) and anhydrous pyridine (1.5 mL) and the reaction mixture, in which a fine precipitate formed after ca. 15 min, was stirred at room temperature for 24 h. Further portions of both methanesulphonic anhydride (1.26 g, 7.29 mmol) and dry pyridine (1.5 mL) were added after ca. 2 h. The mixture was then extracted with water (5 × 50 mL), dried ($MgSO_4$) and clarified with decolourising carbon. Removal of the solvent under reduced pressure dissolution in hot methanol, partial concentration under reduced pressure and cooling, furnished the mesylate **28** as a white crystalline solid (0.86 g). A second crop (0.24 g) was obtained by further concentration, seeding and refrigeration of the mother liquors to give a total yield of **28** of 1.10 g (68%). Recrystallisation of a portion from methanol furnished the pure ester m.p. 88.5-89°C with decomposition. Found: C, 69.01; H, 6.79. $C_{19}H_{22}O_3S$ requires C, 69.05; H, 6.71%.); υ_{max} (KBr) / cm-1 3060, 3040, 2960, 2900 (CH), 1600 (C = C, Ar), 1330 and 1180 (S = O); δ_H(360MHz) 7.65 (2H, d, J = 8Hz, 3,4-*H*), 7.38 (2H, t, J = 8Hz, 2,5-*H*), 7.25 (2H, d, J = 7Hz, 1,6-*H*), 5.04 (1H, t, J_{BD} = 4.7Hz, 9-H_D), 2.90 (2H, br s, 7,11-H_A), 2.52 (2H, dt, J_{AB} = ~3Hz, J_{CB} = 16.6Hz, J_{DB} = 4.7Hz, 8,10-H_B), 2.20 (2H, d, JBC = 16.6Hz, J_{AC} = ~2Hz, 8,10-H_C), 1.73 (3H, s, OSO_2CH_3), 1.31 (3H, s, 13-CH_3), 0.81 (3H, s, 14-CH_3).

9a-Azido-8,9,10,11α-tetrahydro-7αH-7,11-methano-12,12-dimethylcyclo-octa[de]naphthalene (**29**). A mixture of the

mesylate **28** (1·0 g, 3·03 mmol), sodium azide (0·40 g, 6·15 mmol) and anhydrous DMF (10 mL) was heated in an oil bath at 80°C for 3 h. Toluene (100 mL) was then added and the reaction mixture extracted with water (3 × 100 mL). The organic layer was separated, dried ($MgSO_4$), clarified with decolourising carbon and concentrated under reduced pressure to give a pale green oil (0·75 g) which was purified by chromatography over silica with light petroleum as eluent to furnish the azide **29** as a white solid (0·56 g, 67%). Recrystallisation of a portion from methanol gave the azide as needles, m.p. 51–52°C. Found: C, 77·52; H, 6·98; N, 15·01; M+, 277·1579. $C_{18}H_{19}N_3$ requires C, 77·93; H, 6·91; N, 15·16%; M+, 277·1579.); υ_{max} (KBr)/cm^{-1} 3060, 3040, 2960, 2900 (CH), 2100 (N=N=N) and 1600 (C=C, Ar); δ_H(360 MHz) 7·70 (2H, d, J = 8 Hz, 3,4-*H*), 7·41 (2H, t, J = 8 Hz, 2,5-*H*), 7·23 (2H, d, J = 7 Hz, 1,6-*H*), 2·95 (2H, br s, 7,11-H_A), 2·92 (1H, tt, J_{BD} = 12·1 Hz, J_{CD} = 5·6 Hz, 9-H_D), 2·21 (2H, td, J_{AB} = 2·8 Hz, J_{CB} = 12·1 Hz, J_{DB} = 12·1 Hz, 8,10-H_B), 1·89 (2H, dq, J_{AC} = ~2 Hz, J_{BC} = 12·1 Hz, J_{DC} = 5·6 Hz, 8,10-H_C), 1·38 (3H, s, 13-CH_3), 0·75 (3H, s, 14-CH_3).

8,9,10,11α-Tetrahydro-7αH-7,11-methano-12,12-dimethylcycloocta[de]-naphthyl-9β-amine (**30**). A solution of the azide **29** (280 mg, 1·0 mmol) in absolute ethanol (15 mL) containing 10% palladium on charcoal (50 mg) was shaken in an atomosphere of hydrogen for 3 h. The catalyst was removed by filtration through a bed of Celite and the filtrate concentrated under reduced pressure to give the amine **30** as a white semi-solid (245 mg, 97%). Crystallization of a portion from ether afforded an analytical sample as needles, m.p. 74–76°C. Found: C, 85·46; H, 8·44; N, 5·39; M^+, 251·1674. $C_{18}H_{21}N$ requires C, 86·00; H, 8·43; N, 5·57%; M+, 251·1674.); υ_{max} (KBr)/cm^{-1} 3340 (NH), 3060, 3040, 3010, 2990, 2940, 2890 (CH), 1600 (C=C, Ar), 1390, 840 and 785; δ_H(360 MHz) 7·66 (2H, d, J = 8 Hz, 3,4-*H*), 7·39 (2H, t, J = 8 Hz, 2,5-*H*), 7·20 (2H, d, J = 7 Hz, 1,6-*H*), 2·87 (2H, br s, 7,11-H_A), 2·29 (1H, tt, J_{BD} = 12·1 Hz, J_{CD} = 4·5 Hz, 9-H_D), 1·99 (2H, td, J_{AB} = 2·8 Hz, J_{CB} = 11·3 Hz, J_{DB} = 12·1 Hz, 8,10-H_B), 1·75 (2H, dq, J_{AC} = ~2 Hz, JBC = 11·3 Hz, JDC = 4·5 Hz, 8,10-H_C), 1·62 (2H, s, NH_2) 1·36 (3H, s, 13-CH_3), 0·74 (3H, s, 14-CH_3).

Biological assays

The PKC inhibitory potency of the tetracyclic analogues was evaluated at the Wellcome Research Laboratories through the kindness of Dr L. Garland using an assay system based on the mixed lipid micelle assay of Hannan et al (1985) but using phorbol 12, 13-dibutyrate (PDBu) in place of *sn*-1,2-diacylglycerol and phosphocellulose anion exchange papers as opposed to acid precipitation for isolation of the phosphorylated substrate. All the compounds tested lacked inhibitory potency.

Results and Discussion

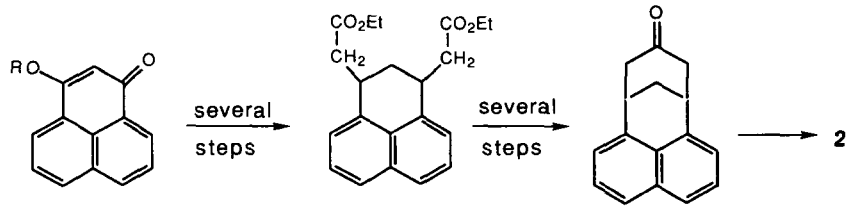
Our original objective was the synthesis of the parent amine **2** starting from 2,3-dihydrophenalene-1,3-dione **3a** readily available (Geissman & Morris 1944) from the reaction of naphthalic anhydride and diethyl malonate in the presence of anhydrous zinc chloride and proceeding sequentially (Scheme 1) by Reformatsky additions to both carbonyl groups, reductive removal of the hydroxyl functions, Dieckmann cyclization to the tetracyclic ketone **4** followed finally by the introduction of the 9-amino group.

The dione (Geissman & Morris 1944) **3a**, a high-melting, sparingly-soluble, deep yellow solid existing in a 'trans-fixed' non-chelated enolic form (Karlsone et al 1965) was recovered unchanged after prolonged treatment with a solution of ethyl bromozincacetate in dimethoxymethane (Cur & Gaudemar 1969) followed by an acidic work up. Its *O*-methyl and *O*-ethyl enolic ethers (Errera 1911) **3b** and **3c** proved to be similarly unreactive but the corresponding enolic acetate (Eistert et al 1968) **3d** gave a complex mixture containing the 3-oxo- and 3-acetoxy unsaturated esters **5** and **6** as shown by ions at m/e 266 and 308, respectively in its mass spectrum. Reaction with ethyl lithioacetate at −70°C was similarly unsuccessful. Condensation of the dione **3a** with (carbethoxymethylene) triphenylphosphorane gave a black tar.

A different potentially attractive route to the required tetracyclic system viz. base—catalysed tandem intermolecular Michael-Claisen reactions of ethyl acetoacetate with the known (Shein 1959) phenalene-1-one **7** to yield the keto-ester **8** was next examined. Once again the atypical unreactivity of the carbonyl function encountered in the earlier series showed itself. Many attempts under a variety of conditions proved fruitless despite the similar reaction undergone by the similarly conjugated benzylideneacetone, 4-phenyl-but-3-en-2-one (Rosenmund et al 1954) with diethyl malonate.

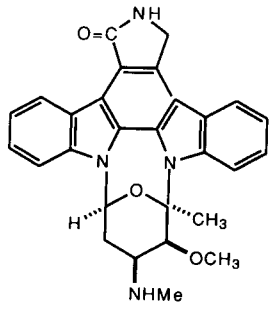
We now returned to the original dione system starting with the known *gem*-dichloro and dimethyl derivatives of **3a** on the assumption that in the absence of conjugation, normal activity would be restored.

In the event both ketones reacted smoothly at room



3a R=H
3b R=Me
3c R=Et
3d R=MeCO

Scheme 1.



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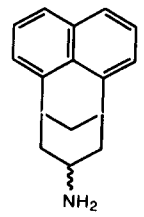

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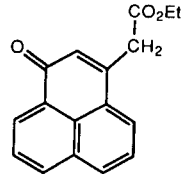

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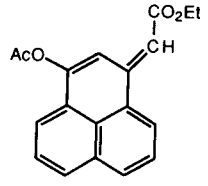

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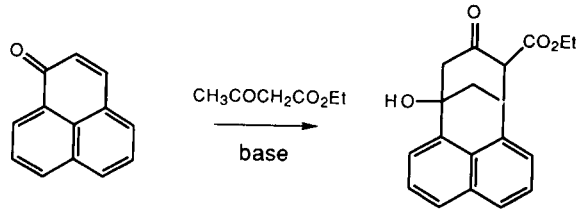
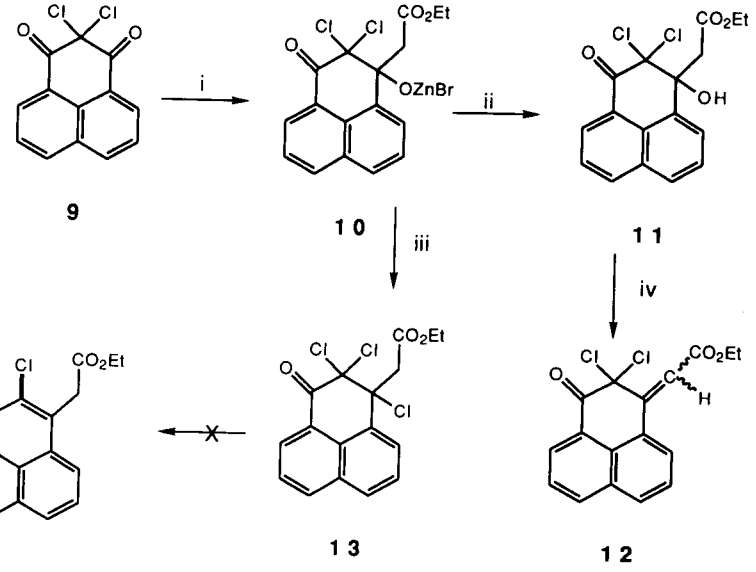
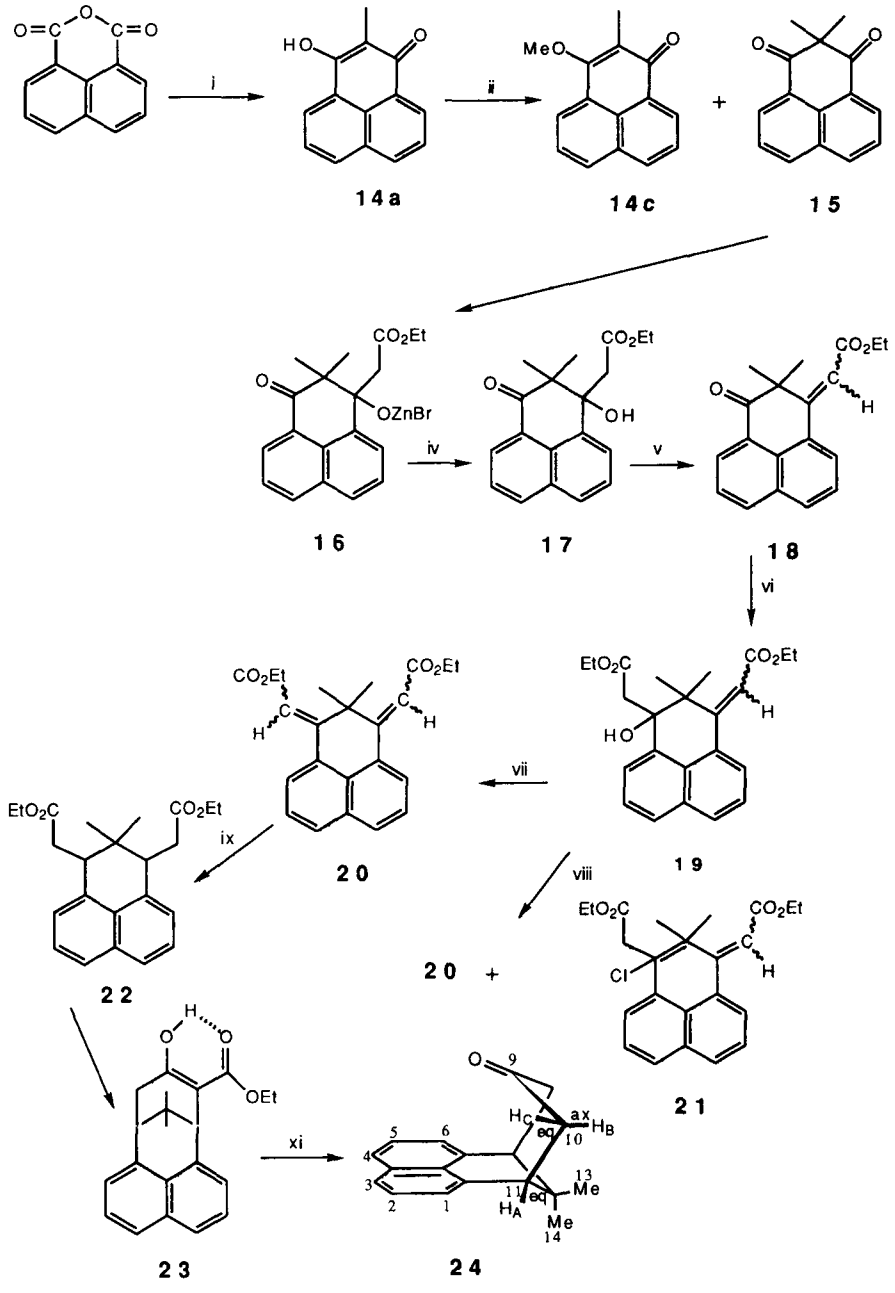


7 8



9 10 11 12 13

Scheme 2. Reagents: i–ii. Br2nCH$_2$CO$_2$Et-THF, 40 min, H$_2$O-H$^+$; iii. SOCl$_2$-C$_6$H$_6$, 50°C, 70 min; iv. P$_2$O$_5$-celite-C$_6$H$_6$, 90°C, 1h.



Scheme 3. Reagents: i. $MeCH(CO_2Et)_2$-$ZnCl_2$, 145–180°C, 6 h, NaOH, H^+; ii. MeONa-MeOH-MeI-$MeCOMe_3$, 3 h, KOH, SiO_2-$CHCl_3$; iii. $BrZnCH_2CO_2Et$-THF, 30 min; iv. H^+-CH_2Cl_2 (or PhMe); v. P_2O_5-celite-C_6H_6, reflux, 1.5 h; vi. $BrZnCH_2CO_2Et$-THF, 5 min, H^+; vii. P_2O_5-celite-C_6H_6, reflux, 1.5 h; viii. $SOCl_2$-C_5H_5N-C_6H_6, 2–10°C, 1.5 h, H^+; ix. Pd-C (10%)-H_2-EtOH; x. $KOBu^t$-PhMe, 50°C, 30 min, H^+; xi. H^+-$MeCO_2H$, reflux, 10 h.

temperature in THF with the Reformatsky reagent with immediate precipitation of zinc complexes which on hydrolysis furnished the mono-β-hydroxyesters **11** and **17**, respectively in high yield as the sole product. Attempts to obtain the corresponding diesters under forcing conditions, including the employment of DMF as solvent to solubilize the zinc complex at 130°C, proved abortive.

Dehydration of the β-hydroxyester **11** with phosphorus pentoxide in benzene (Scheme 2) led to a mixture of the *cis* and *trans* isomers of the α,β-unsaturated ester **12** as evidenced by the ^1H NMR spectrum which showed vinylic protons at d 7·10 and 6·57 in the ratio 5 : 3. Attempts to scale up this reaction were unsuccessful. Attempted dehydration of the zinc complex **10** using thionyl chloride alone or with pyridine gave the highly crystalline trichloroester **13** in reasonable yield. This was unexpected (Kon & Nargund 1932) and would seem to be a characteristic of this system since a similar result was obtained for the 2, 2-dimethyl series (see later). Attempts to convert the trichloroester **13** to the monochloro unsaturated ester using zinc dust and acetic acid gave a complex mixture of products. This failure led to this line of investigation being discontinued in preference to more promising parallel studies in the 2,2-dimethyl series.

Attempts to synthesise the known (Geissman & Morris

1944) 2,2-dimethyldione **15** from the parent dione **3a** presented difficulties (Scheme 3). The method of Geissman & Morris (1944) involved further methylation of the mono-methyl diketone **14a** prepared from **3a** under high pressure conditions. Using methyl iodide and sodium ethoxide a mixture of the C,O-dimethyl enol ether **14c** and the required dimethyldione **15** was obtained which was not easy to separate. We found that a modified approach from **3a** using two molar equivalents of methyl iodide and base in DMF gave a mixture of dimethyl-dione **15**, the *O*-methyl enol ether **14b** and the C,O-dimethyl enol ether **14c** from which, on chromatography, a fairly low yield of **15** was obtained. Neither of these processes, however, would have been capable of supplying the required dimethyldione **15** in quantity.

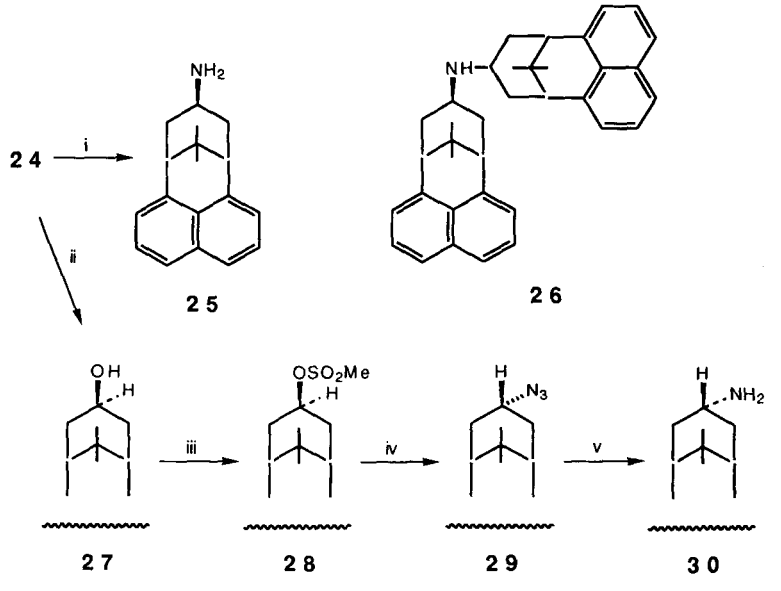
An alternative new process capable of scale-up and based on the original synthesis of the parent dione **3a** but employing diethyl methylmalonate furnished, in high yield, the C-methyldione **14a** converted readily by the reaction described above to a mixture of the required 2,2-dimethyldione **15** and the corresponding C, O-dimethyl derivative **14c**. Treatment of this mixture with the Reformatsky reagent deposited the insoluble zinc adduct 16, the enol-methylether **14c**, as anticipated from earlier work, being unattacked. The zinc adduct was removed and hydrolysed to the mono-β-hydroxyester **17** and the unreacted C,O-dimethyl enol ether **14c** recovered and hydrolysed to the starting material **14a**. The crude yield of the mono-β-hydroxyester **17** was 74% and since the Reformatsky reaction gave quantitative yields with pure **15** this suggests that **15** and the C,O-dimethyl enol ether **14c** exist in the mixture in the ratio 3 : 1.

The inability of the Reformatsky reaction on **15** to give the di addition product, as noted previously for **9**, suggests that the unreacted carbonyl group is shielded from attack by disubstitution at the 2- and 3- positions, a view reinforced by lack of reactivity of the β-hydroxyester **17** with ethyl lithioacetate at $-70°C$ and successful reaction with ethyl bromozincacetate when steric hindrance was alleviated in the more planar α,β-unsaturated ester **18** (see below).

The β-hydroxyester **17** was readily dehydrated by phosphorous pentoxide to give **18** which reacted with ethyl bromozincacetate to give the β-hydroxy diester **19** in good yield. Repetition of the dehydration reaction gave the doubly α,β-unsaturated diester **20**. Alternative use of thionyl chloride and pyridine gave a mixture of **20** and the β-chloroester **21** (c.f. 2,2-dichloro- series) in a 1 : 2 ratio as evidenced by the presence of a $-CH_2-$ group and the different chemical shifts for =CH and 2,2-dimethyl protons in the ^1H NMR. Separation of the two components could not be achieved.

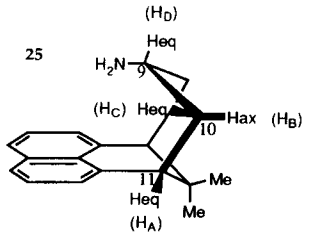
Hydrogenation of **20** gave the saturated diester **22** in good yield whereas a moderate quantity was obtained from the mixture of **20** and **21** by seeding of the crude hydrogenated mixture. Cyclization of **22** using potassium *tert*-butoxide gave the β-ketoester **23** in 63% yield which was quantitatively decarboxylated on heating with aqueous acid to the required intermediary tetracyclic ketone **24**.

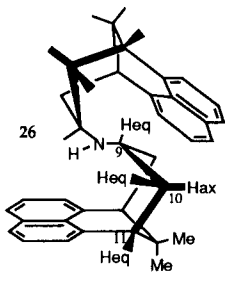
Interestingly, the β-ketoester **23** existed as the 'enol chelate' as evidenced by the IR and ^1H NMR spectra and, furthermore, the $-CH_2-$(ester) protons were coupled to give a quadruple quartet due to the adjacent chiral centre. A Leuckart reaction on the tetracyclic ketone **24** (Scheme 4) with formamide-formic acid followed by acid hydrolysis gave a low yield of a single epimer **25** and a dimer **26** in greater yield. The formation of secondary amine products in this reaction is usually diminished (Moore 1950) by use of a large excess of formamide as followed here. The stereospecificity of this reaction varies since steroidal 3- and 17-ketones give predominantly one isomer (Sauers 1958; Davis et al 1966) whereas certain substituted cyclohexanone systems give mixtures of epimers (Noyce & Batchelor 1952; Hey et al 1967; Coe et al 1968). ^1H NMR studies (see later) showed that **25** had a chair conformation with an axial primary amine.



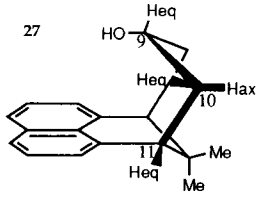
Scheme 4. Reagents: i. HCO_2H-$HCONH_2$, reflux, 120 h, SiO_2-$CHCl_3$; ii. $LiAlH_4$-THF, H_2O - NaOH; iii. $(MeSO_2)_2O$-CH_2Cl_2-C_2H_5N, 24 h; iv. NaN_3-DMF, 80°C, 3 h; v. Pd-C (10%)-H_2-EtOH, 3 h.

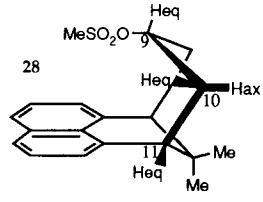
Table 1. NMR data for axial substituted tetracyclic analogues.





Proton[a]	δ (multiplet)[b]	Protons	J (Hz)	Proton[a]	δ (multiplet)	Protons	J (Hz)
9eq	3.10 (t)	9eq10ax	~6.2[b]	9eq	2.09 (t)	9eq10ax	~5.6[b]
10ax	2.57 (dt)	9eq10eq	small[c]	10ax	1.69 (dt)	9eq10eq	small[c]
10eq	1.78 (d)	10ax10eq	14.5	10eq	0.68 (d)	10ax10eq	14.3
11eq	2.85br (s)	10ax11eq	~3[d]	11eq	2.40br (s)	10ax11eq	~4[d]
		10eq11eq	small[c]			10eq11eq	small[c]





Proton[a]	δ (multiplet)[b]	Protons	J (Hz)	Proton[a]	δ (multiplet)	Protons	J (Hz)
9eq	3.92 (t)	9eq10ax	~5.2[b]	9eq	5.04 (t)	9eq10ax	~4.7[b]
10ax	2.50 (dt)	9eq10eq	small[c]	10ax	2.52 (dt)	9eq10eq	small[c]
10eq	2.00 (dd)	10ax10eq	14.7	10eq	2.20 (dd)	10ax10eq	16.6
11eq	2.87 (t)	10ax11eq	~3[d]	11eq	2.90br (s)	10ax11eq	~3[d]
		10eq11eq	small[c]			10eq11eq	small[c]

[a] Only cyclohexane ring protons included. [b] Approximated from the 9eq triplet assuming a very small coupling for 9eq10eq. [c] Negligible splitting obscured by line broadening. [d] Inferred from approximate measurements of multiplet.

Synthesis of the equatorial substituted amine **30** was achieved as outlined in Scheme 4, the stereochemistry of the products being confirmed by ^{1}H NMR studies. Reaction of the axial methanesulphonate **28** with azide ion occurred with the expected inversion (Biffin et al 1971) to the equatorial azide **29**.

NMR studies

The parent ketone **24** has a characteristic ^{1}H NMR spectrum (see **24** and **25** for numbering) with the equatorial proton on C-11 (H-11eq) appearing as a doublet, being coupled to the axial proton on C-10 (H-10ax) (J = 5·0 Hz) but having a negligible coupling to H-10eq. H-10ax appears as a double doublet which arises from coupling to H-11eq and H-10eq (J = 16·1 Hz) whereas H-10eq consequently appears as a doublet. These data are consistent with the cyclohexane ring adopting a distorted chair conformation with the H-11eq-C-11-C-10-H-10eq dihedral angle increased from 60° resulting in negligible H-11eq-H-10eq vicinal coupling. X-ray crystallography (Hughes & Hursthouse 1995) confirmed these conclusions and gave a dihedral angle of 67·74°.

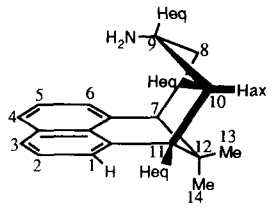
Examination of the ^{1}H NMR spectra for the compounds **25**–30 revealed two distinct multiplet patterns. The primary

Table 2. Decoupling experiments on the primary amine **25**.

	Observed Muliplets[a]			
Decoupled Proton	9eq (t)	10ax (dt)	10eq (d)	11eq (br s)
9eq	-	**dd**	d	br s
10ax	**s**	-	**s**	**s (sharp)**
10eq	t	**t**	-	**d**
11eq	t	**dd**	**d (sharp)**	-

[a] Changed multiplets are indicated in bold type

Table 3. Nuclear Overhauser effect experiments on the primary amine **25**.



	N.O.E. (%) Observed for Proton Multiplets						
Irradiated Proton	1	9eq	10eq	10ax	11eq	13	14
9eq	-	-	1	2.5	-	-	-
10eq	-	small[a]	-	14	3	-	-
10ax	-	7	13	-	2	2.5	-
11eq	7	-	3	1	-	1	1.5
13	-	-	-	6	2	-	-

[a] immeasurably small integral

amine **25** displayed a set of proton mutiplets also characteristic for the alcohol **27**, mesylate ester **28** and secondary amine **26** which were consistent with a distorted chair conformation and axial group substitution on C-9 (Table 1). Decoupling experiments (Table 2) performed on the primary amine **25** verified the conclusions shown in Table 1.

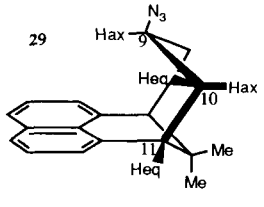
To verify the chair conformation with an axial amino group a series of Nuclear Overhauser effect experiments were also conducted on the primary amine **25** (Table 3). The results confirm the assigned chair conformation and furthermore unequivocally confirm all of the multiplets in the ^{1}H NMR spectrum. Taken together, these data are consistent with the primary amine **25**, secondary amine **26**, alcohol **27** and mesylate ester **28** having a cyclohexane ring in a chair conformation with an axial substituent. The cyclohexane ring is distorted from an ideal chair such that the axial substituent is lifted further above the plane of the naphthalene ring with the dihedral angles H-9eq-C-9-C-10-H-10eq and H-10eq-C-10-C-11-H-11eq increased from 60°. X-ray crystallography (Hughes & Hursthouse 1995) on **26** confirmed these conclusions and gave dihedral angles of 78·74° and 65·61° respectively. The other primary amine **30** and azide **29** both display a common but different multiplet pattern. Given the known inversion of configuration that accompanies azide nucleophilic substitution, it is reasonable to presume that both compounds have a similar chair cyclohexane conformation but contain equatorial substituents i.e. the primary amines **25** and **30** are epimers. This presumption is verified by their ^{1}H NMR spectra, the data for which is summarised in Table 4.

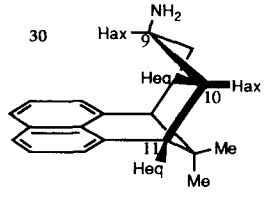
Biological results

The tetracyclic compounds did not inhibit PKC despite the good fit seen on superimposition of **2** and the potent inhibitor staurosporine **1** (Fig. 1). Possible explanations for this lack of inhibitory potency are now considered.

Dimethyl substitution at C-12 in the amine **25**, although necessary for synthetic reasons, further reduces its similarity to staurosporine by effectively reducing the structural planarity and increasing hydrophobicity. However planarity of

Table 4. NMA data for equatorial substitutional tretracyclic analogues.





Proton[a]	δ (multiplet)	Protons	J (Hz)	Proton[a]	δ (multiplet)	Protons	J (Hz)
9ax	2.92 (tt)	9ax10ax	12.1	9ax	2.29 (tt)	9ax10ax	12.1
10ax	2.21 (td)	9ax10eq	5.6	10ax	1.99 (td)	9ax10eq	4.5
10eq	1.89 (dq)	10ax10eq	12.1	10eq	1.75 (dq)	10ax10eq	11.3
11eq	2.95br (s)	10ax11eq	2.8	11eq	2.87br (s)	10ax11eq	2.8
		10eq11eq	small[b]			10eq11eq	small[b]

[a] Only cyclohexane ring protons included. [b] Very small coupling obscured by line broadening.

staurosporine is clearly not essential since non-planar bisindoylmaleimides (Davis et al 1992b), e.g. RO31—8425, IC50 = 7·6 nM, have equal potency which suggests some degree of structural tolerance in the hydrophobic pocket of the binding site.

Bisindoylmaleimides (Davis et al 1992a, b) 29,30 are potent inhibitors and the maleimide moiety mimics the lactam ring of staurosporine suggesting that the latter is an important binding site; these types of function are absent in the tetracyclic compound **25**.

NMR spectroscopy (Davis et al 1991) shows that the bioactive conformation of the sugar moiety of staurosporine may be either a chair, which is the solution conformation and crystal structure of the amino sugar free base, or a boat, which is the solution conformation of the protonated form. However, given the low basicity of the amine ($pK_a = 5·3$) it has been argued that binding may occur through the chair form at physiological pH. However it seems more likely that the protonated form (boat) is the active form since the potent bisindoylmaleimides possessing a flexible primary amine side chain would be completely ionized at physiological pH. Furthermore in this series where the side chain is conformationally restricted (Davis et al 1992b) in a ring e.g. RO31—8425 graphics modelling (Bit et al 1993) showed that these compounds modelled the boat conformation of staurosporing more accurately than the chair form which further suggests that the boat form of staurosporine is the bioactive form.

On the assumption that the protonated form of staurosporine is the active form then the lack of potency of **25** may be, in part, due to its shown inability to undergo the chair to boat interconversion on protonation as a result of steric hindrance by the 12-methyl group.

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