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CRYSTALLINE CONSTITUENTS FROM EUPHORBIACEAE—XIII[a]

THE STRUCTURE OF A NEW TRITERPENE FROM *EUPHORBIA NERIFOLIA* L.

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Abstract—From the leaves and stems of *Euphorbia nerifolia* Linn., a new triterpene was isolated besides friedelan-3α- and 3β-ols, and taraxerol. From a study of its chemical reactions and spectral data, the new triterpene was shown to be glut-5(10)-en-1-one(**3**).

Euphorbia nerifolia Linn.[1] (Euphorbiaceae) is a shrub with thick stems and large oval-shaped leaves. Frequently, the leaves are used indigenously in the treatment of bronchial and intestinal disorders. From its stems, Row and Anjaneyulu[2] reported a new triterpene besides friedelan-3α- and 3β-ols and taraxerol. During a re-examination of this plant, this triterpene was also found in the leaves. The present paper describes chemical and spectroscopic evidence leading to the proposal of structure **3** for this new triterpene.

The new compound is a pentacyclic triterpene containing an α:β-unsaturated ketonic function ($C_{30}H_{48}O$; M^+ 424; m.p. 312–14°, $(\alpha)_D + 50°$, pink colour in L-B reaction and pale yellow in TNM Test). The triterpene does not respond to Zimmermann's test suggesting that it is not likely to be a 3-ketone.[3] But the ready formation of 2,4-dinitrophenylhydrazone (m.p. 281° (dec)) and also a crystalline benzylidene derivative (m.p. 300–02°, $(\alpha)_D + 11°$ (λ_{max}^{EtOH} 228, 292 nm, log ϵ 4·31, 4·66) suggest that it should have a $-CH_2-CO-$ group located in a fairly accessible position. The NMR also shows a multiplet at τ 7·60–7·80 integrating for the two protons of $-CH_2-CO-$ group.

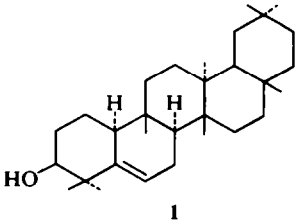
A significant feature of the NMR spectrum of the new triterpene is the absence of any olefinic protons suggesting that the double bond is tetrasubstituted, and the three weak bands at 880, 840 and 810 cm^{-1} in its IR spectrum confirm this conclusion.

The clue for the basic skeleton of this new triterpene was secured by a study of the hydrocarbon obtained by reduction with platinum oxide catalyst, in glacial acetic acid ($C_{30}H_{50}$, M^+ 410) m.p. 228–30°, $(\alpha)_D - 40°$), which obviously relates to a pentacyclic triterpene with a tetra-substituted double bond. After much search, it was found identical in every respect (m.m.p., TLC and IR) with glut 5(10)-ene (**2**),[4,5] secured by the conversion of glut-5-en-3β-ol (**1**) from *Euphorbia royleana*,[6] according to the procedure of Beaton *et al.*[4] This was further confirmed by the conversion of the reduced new triterpene (**2**) into an equilibrium mixture of 18α-olean-12-ene and olean-13(18)-ene.[4]

Having thus identified the basic skeleton of the new triterpene as glut-5(10)-ene (**2**), evidence is next gathered to fix the α:β-unsaturated ketonic system (ν_{max} 1670 cm^{-1}) in the new triterpene which can have two possible structures **3** and **4**.

The new triterpene exhibited a somewhat anomalous UV absorption spectrum in ethanol, which showed only one peak at 218 nm (log ϵ 3·87).

[a]Part XII. *Tetrahedron* **29**, 1291 (1973)



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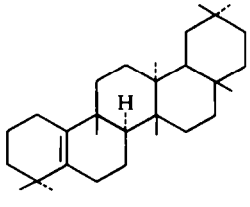

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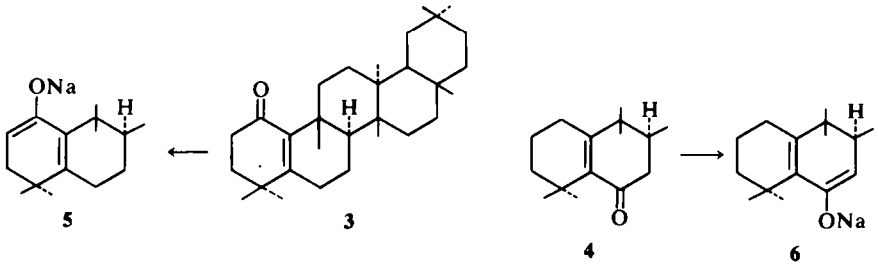


5 3 4 6

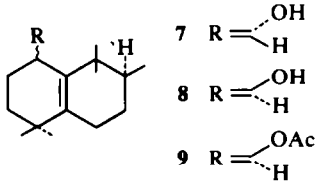
However, on addition of sodium ethoxide a new prominent peak appeared at 274 nm, suggesting the formation of an enol. The new peak at 274 nm is consistent with the formation of a homoannular diene system (**5** and **6**). It can be rationalised, if the newly formed double bond conjugates with the existing 5(10)-double bond in the parent triterpene, thus limiting the choice to the structures **3** and **4**.

Further, the 2,4-dinitrophenylhydrazone of the new triterpene exhibited two prominent peaks at 248 and 381 nm (log ϵ, 4·48, 4·69) confirming an $\alpha:\beta$-unsaturated ketonic system in the triterpene.[7] The anomalous UV absorption of the parent triterpene may probably be due to the non-coplanarity of the carbonyl with the double bond.[7] An examination of the Dreiding models of structures **3** and **4** suggested that the 1-ketone can be non-coplanar when ring A assumes a boat or quasi-boat conformation, while the 6-keto group retains its coplanarity with the double bond. This seems to satisfactorily explain its UV behaviour. Similar instances of non-coplanarity resulting in a hypsochromic shift were noticed in several cyclic systems.[7]

The Dreiding models further suggest a free access to 1-keto group unlike the 6-keto group which is sterically hindered by 4β-axial Me group, thus supporting its facile chemical reactivity with 2,4-dinitrophenyl hydrazine and during catalytic reduction to the hydrocarbon (**2**). These reactions are therefore taken to lend support for the structure **3** for the new triterpene.

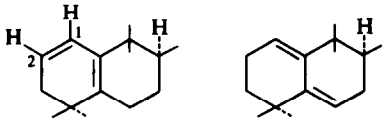
Further support for the structure **3** was derived by a study of its chemical reactions. When the new triterpene was refluxed with LAH in THF, a mixture of alcohols (**7** and **8**), m.p. 268–70°, was formed. Attempts to separate them through their acetates furnished a crystalline acetate, ($C_{32}H_{52}O_2$, M^+ 468), m.p. 238–40°, $(\alpha)_D$ + 28·5°) in 50% yield. The mother liquors contained another isomeric acetate; but it could not be isolated in a pure condition. In the NMR spectrum of the pure crystalline acetate, the acetoxyl appeared at τ 8·03s and its α-hydrogen at τ 5·10 m, which is assignable clearly to the α-axial hydrogen,[8] suggesting that it is 1β-acetate (**9**). It may be mentioned here that the spectrum contained no olefinic protons, as in the parent triterpene.

An interesting reaction was observed when an attempt was made to separate the mixture (**7** and **8**)



through their benzoates. When the mixture was treated with benzoyl chloride in pyridine at 100° for $3\frac{1}{2}$ hr, no benzoylation took place; instead two conjugated dienes, A (**10**) and B (**11**), were secured.

Diene A (**10**), $C_{30}H_{48}$, m.p. 260–62°, $(\alpha)_D$ + 15·5°, is a homoannular diene (λ_{max}^{EtOH} 274 nm (log ϵ 3·89)). In its NMR spectrum (Fig A, 100 MHz, $CDCl_3$), there is a neat quartet centered at τ 4·31, J = 10, 3 Hz, assignable for 2-H. The multiplet between τ 4·57–4·61 also integrates for one proton, assigned for 1-H. The quartet and the multiplet together can be analysed for an ABX system consisting of the protons 1-H, 2-H and the 3β-H. The 3α-H makes a dihedral angle of about 90° and therefore does not couple with either 1-H or 2-H as per Karplus equation.[9] It is probable that 1-H is also coupled with a long distance proton, probably 11-H. A similar complex system of olefinic signals was noticed in saikogenin E diacetate[10] and related compounds.



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Diene B (**11**), $C_{30}H_{48}$, m.p. 162–64°, $(\alpha)_D$ − 19°, has a UV absorption with triple peaks at 233, 238 and 248 nm (log ϵ 4·42, 4·37 and 4·30), characteristic of a heteroannular diene. This absorption is in close agreement with that observed for glut-1(10), 5-dien-3β-yl acetate[11] and simiarenyl acetate.[12] The NMR spectrum of diene B (**11**) (Fig B, 100 MHz, $CDCl_3$) is comparatively simple, and as expected contained two triplets at τ 4·62 and τ 4·82, each integrating for one olefinic hydrogen. Therefore, diene B is glut-1(10), 5-diene (**11**).

Further evidence for structure (**3**) for the new triterpene was sought by a study of the SeO_2 oxidation. When (**3**) was refluxed with SeO_2 in glacial

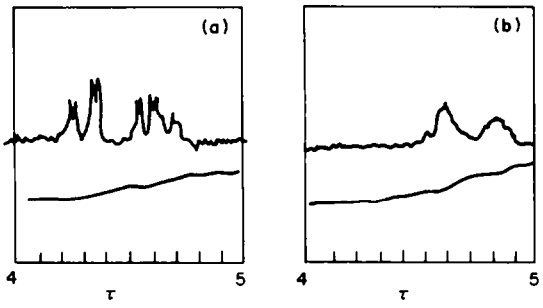

Fig 1.

acetic acid for 3½ hr, it gave (12) as yellow plates, $C_{30}H_{44}O_2$, m.p. 278–80°, $(\alpha)_D$ +111·4°, λ_{max}^{EtOH} 255 nm (log ε 3·20). This new compound 12 did not give any colouration with $FeCl_3$. Its IR spectrum showed strong peaks at 1704 cm^{-1} (6-membered ketone), 1660 and 1600 cm^{-1} (α:β-unsaturated ketone). The NMR spectrum (60 MHz; $CDCl_3$) provided valuable information regarding its structure. One of the eight Me's appeared lower field at τ 8·70 while the remaining seven Me's absorbed between τ 8·83 and 9·05. This shift in the Me signal suggests that the 9β-Me must have shifted to C-10, where it comes under the deshielding influence of the 1-CO group. Another important feature of this spectrum is the appearance of a singlet at τ 4·02 integrating for one olefinic hydrogen. This is clearly assignable to the α- proton of an α:β-unsaturated ketonic system. On the basis of the foregoing information, the structure 12 is proposed for the SeO_2 oxidation product, whose formation could be rationalised as shown in Chart-A below.

The shift of C-9 Me to C-10 is not unknown in literature. Such a shift has been noticed in the transformation of friedel-3-ene to 18α-olean-12-ene and olean-13(18)-ene.[13-15] This shift is further supported by the olefinic hydrogen which appears at τ 4·02 as a singlet and can be located at C-6. If the Me group has not shifted to C-10, and if C-10 carried a hydrogen, the C-6 hydrogen should have given a neat doublet with J = 2 Hz, as in glut-5-en-7-ene.[16] So the singlet at τ 4·02 confirms that there is no hydrogen at C-10, which must, therefore, be occupied by a Me.

The mass spectra of the new triterpene (3), its acetate (9) and the hydrocarbon (2) are now presented in support. The fragment *m/e* 205 is prominent in all the three compounds and forms the base peak in the hydrocarbon (2). It represents D and E ring systems whose formation can be rationalised as in Chart-B. The base peak in the new triterpene (3) is *m/e* 137. The base peak in the acetate (9) is *m/e* 408, produced by the loss of acetic acid molecule. Other abundant ions are *m/e* 205(94), 274(80) and 259(74). The fragmentation pattern is quite similar in all these three compounds and follows the well known pattern of fragmentation noticed in pentacyclic triterpenes.[17,18] The fragmentation is illustrated in Chart-B below.

It may be mentioned here that glut-5(10)-ene (2) is a stable intermediate in the friedelene-oleanene rearrangement.[5] So far neither 2 nor any of its derivatives have been isolated from nature. The isolation of the new triterpene (3) from *E. nerifolia* Linn. is the first instance of the isolation of glut-5(10)-ene (2) derivative from nature. This is also the first reported instance of an oleanane in which the 3-oxygen function is absent, although several members of hopane series, such as zeorin,[19,20] leucotylin,[21] which have no 3-oxygen function were recently isolated from lichens and ferns.

An oxygen function at C-1 is a rarity in β-amyrins or related pentacyclic triterpenes. Glochidonol (1-hydroxy-lupenone) from *Glochidion wrightii* Benth (Euphorbiaceae)[22] and glochidiol (1-hydroxy-lupeol) from *G. hohenckeri*[23] were recorded previously, and new glut-5(10)-en-1-one (3), the new tritterpene, is recorded in *E. nerifolia* Linn. (Euphorbiaceae).

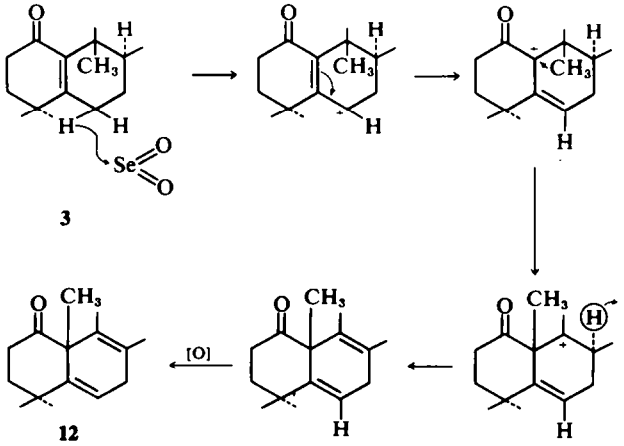

CHART A

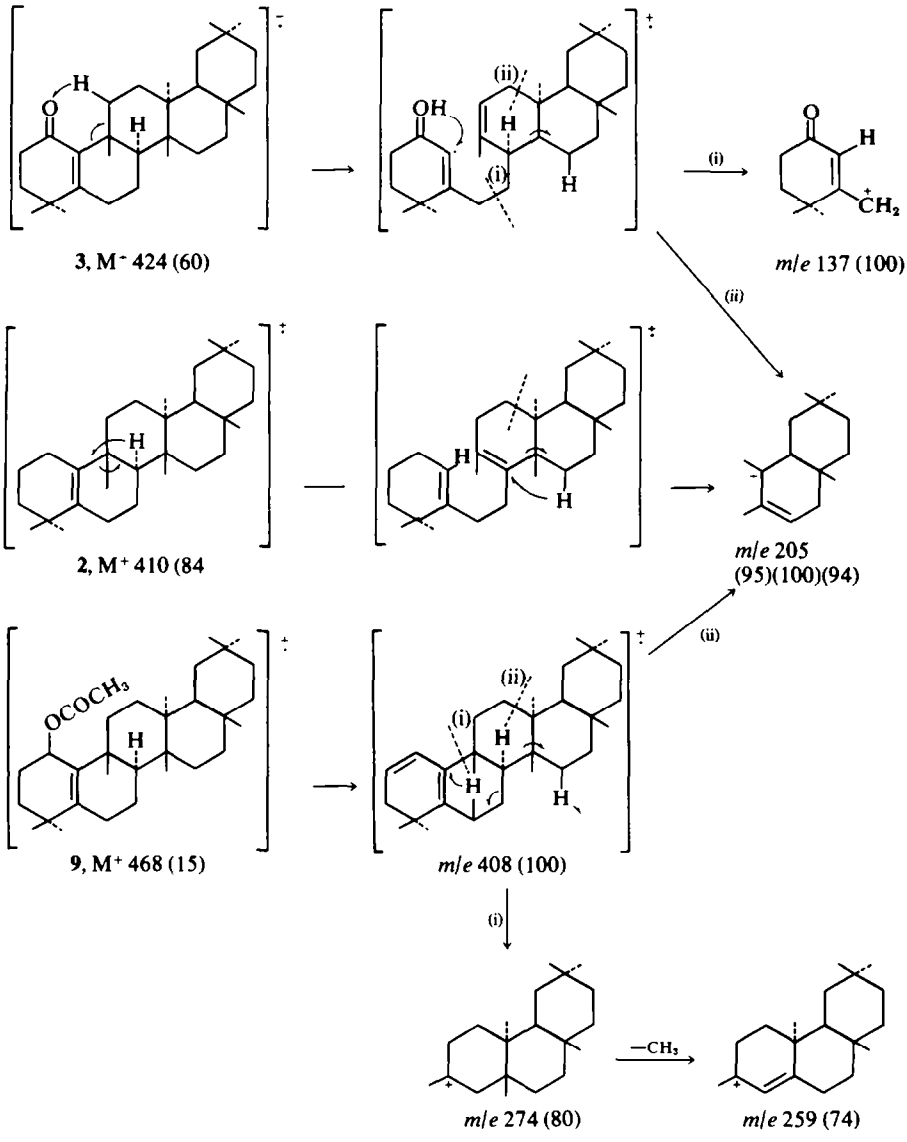

CHART B

EXPERIMENTAL

All the m.p.s are uncorrected. IR spectra were taken on Perkin-Elmer Infracord Spectrophotometer (237) and UV spectra on Beckmann-DB-G Spectrophotometer in EtOH. All the optical rotations were taken in $CHCl_3$. Silica gel C was used for TLC with benzene-$CHCl_3$ (1 : 1) as the solvent system.

The triterpenes of the leaves of E. nerifolia Linn

The sun-dry leaf powder (2Kg) was continuously extracted with hexane, ether and alcohol successively.

The dark green coloured hexane extract (5 L) when concentrated to 500 ml, deposited a colourless solid (3g), m.p. 264–70° (Fraction I). The filtrate upon complete removal of the solvent deposited a wax-like compound (30g Fraction II). The green coloured ether and alcohol extracts did not furnish any more crystalline compounds; they were, therefore, rejected.

Examination of fraction I. Fraction I (3g) showed 3 spots on TLC. On fractional crystallisation from $CHCl_3$-MeOH, compound A separated as colourless shining prisms, m.p. 308–10° (100 mg). A further crystallisation from the same solvent raised the m.p. 312–14°.

The residue from compound A was dissolved in benzene (10 ml), passed over a column of alumina (30×2·5 cm) and eluted with benzene. The first three fractions (3× 100 ml) gave colourless crystals of compound B, m.p. 280–2°, (1 g). The last three fractions gave colourless needles of compound C, m.p. 300–1° (400 mg).

Examination of fraction II. Fraction II (30 g) contained large quantities of waxes and these were removed by their sparing solubility in MeOH. The solvent was removed and

the residue fractionally crystallised from $CHCl_3$-MeOH to yield compound B, (200 mg) and compound D, (600 mg).

The triterpenes from the stems of E. nerifolia Linn

The stems were also extracted similarly and the yields of the products from 1·5 Kg were as follows: Compound A (225 mg) compound B (400 mg) and compound D (400 mg).

Compound B crystallised from benzene as colourless prisms, m.p. 283–84°, $(\alpha)_D$ + 15°, and it was identified as friedelan-3β-ol (m.m.p. and IR).

Compound C crystallised from benzene as colourless needles, m.p. 302–03°, $(\alpha)_D$ + 20°, and it was identified as friedelan-3α-ol (m.m.p. and IR).

Compound D after several crystallisations from $CHCl_3$-MeOH and finally from benzene came out as colourless needles, m.p. 282–83°, $(\alpha)_D \pm 0°$, identical with authentic taraxerol (m.m.p. and IR).

Compound A

The new triterpene, Glut-5(10)-en-1-one (3). Compound A crystallised from $CHCl_3$EtOAc as colourless elongated plates, m.p. 312–14°, $(\alpha)_D$ + 50° (*c*, 1·0); ν_{max}^{Nujol} 1670, 1120, 880, 840 and 810 cm^{-1}; λ_{max} 218 nm (log ϵ 3·87), shifted to 274 nm on addition of NaOEt gave a pink colour in L-B reaction and a very pale yellow colour with TNM, R_f value 0·65. (Found: C, 84·78; H, 11·58; $C_{30}H_{48}O$ requires: C, 84·84; H, 11·39%; M^+ 424(60), 409(8), 274(8), 259(5), 205(95) and 137(100); NMR, 60 MHz, $CDCl_3$; τ Values: 8·75, 8·78, 8·90, 8·93, 8·97, 9·03 (8 × CH_3).

The 2,4-dinitrophenylhydrazone crystallised as orange needles from $CHCl_3$-MeOH; m.p. 281° (dec); $\nu_{max}^{CHCl_3}$ 3320, 1630 and 1600 cm^{-1}. (Found: C, 69·38; H, 8·11; $C_{36}H_{52}O_4N_4.1H_2O$ requires: C, 69·42; H, 7·84%).

Benzylidene derivative of compound A (3)

Compound A (3; 100 mg) in benzaldehyde (2 ml) was refluxed in an oil bath for 4 h. The mixture was worked up in the usual way, and the product crystallised from $CHCl_3$-MeOH as colourless needles, (50 mg), m.p. 300–02°, $(\alpha)_D$ + 11° (*c*, 0·5). λ_{max} 228, 292 nm (log ϵ 4·31, 4·66). R_f value 0·83. (Found: C, 86·31; H, 10·02; $C_{37}H_{52}O$ requires: C, 86·66; H, 10·22%).

Hydrogenation of compound A

Isolation of glut-5(10)-ene (2). Compound A (3; 100 mg) dissolved in HOAc (50 ml) and cyclohexane (20 ml) was shaken with PtO_2 (80 mg) in presence of H_2 at room temp and pressure. After the absorption of H_2 was complete (1 h), the catalyst was filtered off, the filtrate was diluted with water and extracted with ether. The ether layer on usual working up, gave a product which crystallised from EtOAc as colourless plates, (60 mg), m.p. 228–30°, $(\alpha)_D$ − 40° (*c*, 1·0), undepressed when mixed with a sample of (2) prepared from glut-5-en-3β-ol, following the method of Beaton *et al.*[4] (Found: C, 87·68; H, 11·82; $C_{30}H_{50}$ requires: C, 87·73; H, 12·27%); M^+ 410(84), 395(35), 274(8), 259(7), 205(100).

Acid isomerisation of reduced compound (2). To the above hydrocarbon (2; 40 mg) in HOAc (70 ml), conc HCl (20 ml) was added and the mixture was refluxed in an oil bath for 24 h. Crystallisation of the product from $CHCl_3$-MeOH gave the mixture of 18α-olean-12-ene and olean-13(18)-ene as colourless plates, (15 mg), m.p. 182–84°, $(\alpha)_D$ − 18°, undepressed when mixed with a sample prepared from authentic glut-5(10)-ene in a similar way.

LAH reduction of compound A (3). Compound A (3; 120 mg) in dry THF (60 ml) was refluxed for 8 hr with LAH (400 mg). The excess LAH was destroyed by adding EtOAc and the mixture filtered. The filtrate was evaporated and the residue crystallised from $CHCl_3$-EtOAc to give the mixture of 7 and 8 as colourless plates, (80 mg), m.p. 268–70°; ν_{max}^{Nujol} 3500 cm^{-1}; R_f values 0·61, 0·50.

Acetylation of the above alcohol mixture. The dry alcohol mixture (7 and 8) from the above experiment (100 mg) in pyridine (20 ml) and Ac_2O (4 ml) was warmed at 100° for 3 h and diluted with water. The product 9 crystallised from EtOAc as colourless needles, (50 mg), m.p. 238–40°, $(\alpha)_D$ + 28·5° (*c*, 0·95); ν_{max}^{Nujol} 1730 and 1250 cm^{-1}. (Found: C, 81·91; H, 10·96; $C_{32}H_{52}O_2$ requires: C, 81·99; H, 11·18%). M^+ 468(15), 453(6), 409(70), 408(100), 274(80), 259(74), 205(94). NMR, 60 MHz, $CDCl_3$. τ values: 8·85, 8·88, 8·98, 9·03, 9·05, 9·15 (8 × CH_3), 8·03*s* ($OCOCH_3$), 5·10 m (H—C—$OCOCH_3$).

Benzoylation of the alcohol mixture. The alcohol mixture 7 and 8 (350 mg) from the above experiment in pyridine (25 ml) was treated with benzoyl chloride (5 ml) and kept at 100° for 3½ h. The mixture was worked up as usual. The residue fractionally crystallised from $CHCl_3$-EtOH to give Diene A (10; 80 mg), m.p. 256–58°, and Diene B (11; 100 mg), m.p. 158–60°.

Diene A (10) crystallised from $CHCl_3$-EtOH as colourless shining plates, m.p. 260-62°, $(\alpha)_D$ + 15·5° (*c*, 0·41); ν_{max}^{Nujol} 720 cm^{-1}; λ_{max} 274 nm (log ϵ 3·89). (Found: C, 87·72; H, 11·42; $C_{30}H_{48}$ requires: C, 88·16, H, 11·84%); NMR, 100 MHz, $CDCl_3$, τ values: 8·82, 8·87, 8·96, 9·02, 9·07, 9·11 (8 × CH_3), 4·31dd, J = 10, 3 Hz (1H), 4·66 m (1H).

Diene B (11) crystallised from $CHCl_3$-EtOH as colourless needles, m.p. 162–64°, $(\alpha)_D$ − 19° (*c*, 0·68); λ_{max} 233, 238, 248 nm (log ϵ 4·42, 4·37, 4·30). (Found: C, 87·72; H, 11·36; $C_{30}H_{48}$ requires: C, 88·16; H, 11·84%); NMR, 100 MHz, $CDCl_3$, τ values: 8·80, 8·82, 8·93, 8·98, 9·08, 9·22 (8 × CH_3), 4·62t (1H); 4·82t (1H).

SeO_2 *Oxidation of compound A* (3). Compound A (3; 100 mg) was refluxed in glacial HOAc (20 ml) with SeO_2 (80 mg) for 3½ h. Metallic Se was filtered off and the filtrate allowed to cool. Yellow shining plates of (12) separated out, (60 mg). These were filtered and recrystallised from $CHCl_3$-MeOH, m.p. 278–80°, $(\alpha)_D$ + 111·4° (*c*, 1·0); $\nu_{max}^{CHCl_3}$ 1704, 1660, 1600, 1000, 980 and 890 cm^{-1}; λ_{max} 255 nm (log ϵ 3·20). (Found: C, 82·33; H, 10·10; $C_{30}H_{44}O_2$ requires: C, 82·52; H, 10·16%); NMR, 60 MHz, $CDCl_3$, τ values; 8·70 (3H), 8·83, 8·88, 9·02, 9·05, (7 × CH_3), 4·02s (1H).

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