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NOVEL MONOAROMATIC TRITERPENOID HYDROCARBONS OCCURRING IN SEDIMENTS

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Abstract . Three monoaromatic pentacyclic triterpenoids of the lupane, ursane and oleanane series, and a monoaromatic tetracyclic triterpenoid of the lupane series have been synthesised and shown to occur in several geological samples. Such compounds arise by microbial degradation of their respective 3-oxy-precursors.

INTRODUCTION

Aromatic hydrocarbons related to higher plant triterpenoids oxygenated at C-3[1] occur in various geological samples such as petroleums, brown coals or bottom aquatic sediments.[2-9] The structures of these biological markers strongly suggest that aromatisation processes start with ring A, induced by the presence of the oxygenated function, and proceed sequentially towards ring D(E)[3,4] (fig. 1). As an alternative, the original triterpene may initially undergo loss of ring A and subsequently aromatisation from ring B to D(E)[5,6,9] (fig. 1).

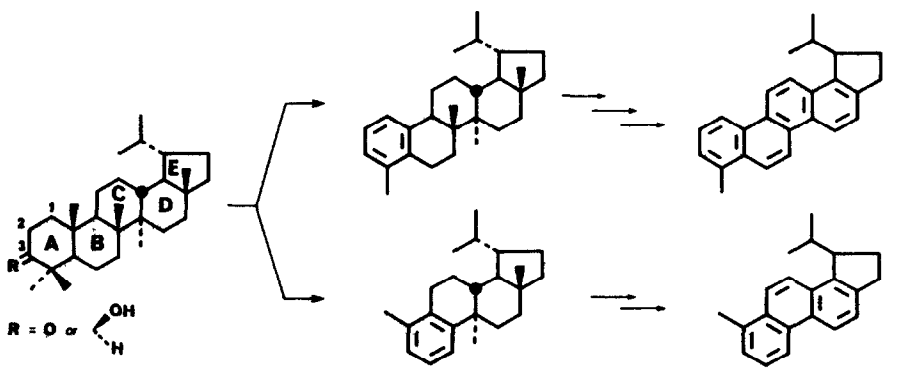

Figure 1: Two of the major postulated aromatisation pathways for the 3-oxy-triterpenes in sediments
Example of the lupane series

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The occurrence of these aromatised terpenoids in very recent sediments implies that these compounds may be produced at the earlier stages of diagenesis, probably *via* microbially mediated reactions.[5] However, if one excepts the case of the allobetulin series[10], no monoaromatic "intermediates" deriving from 3-oxy-triterpenoids have yet been fully identified, although recent studies provide evidence of their presence as major constituents in some surface sediments or brown coals on the basis of mass spectrometry data.[11-14] We wish to report here the synthesis of three pentacyclic A-ring monoaromatic triterpenoids : 24,25-dinorlupa-1,3,5(10)-triene **7a**, 24,25-dinorursa-1,3,5(10),12-tetraene **7b** and 24,25-dinoroleana-1,3,5(10),12-tetraene **7c**, and of a tetracyclic B-ring monoaromatic compound : des-*A*-26-norlupa-5,7,9-triene **10**. These syntheses were aimed, first, at the identification of the compounds in several recent sediments, and second, at the unambiguous characterisation of radioactive monoaromatic products formed during incubation experiments of radiolabelled lupan-3-one and olean-12-en-3β-ol in a pond mud.

RESULTS AND DISCUSSION

*Synthesis of the pentacyclic monoaromatic triterpenoids **7a-c**.*

Figure 2 shows the scheme adopted for the synthesis of the pentacyclic triterpenoids **7a-c**. In the case of **7b** and **7c**, the synthesis was performed on a mixture of their respective starting materials, products being separated at each stage for analysis (see Experimental) ; only the lupane **7a** and ursane **7b** series will be referred to below.

Dehydration of the parent alcohol **1a** with PCl_5[15] furnished a quantitative yield of *A*-neolup-3-ene, which was then isomerised to *A*-neolup-3(5)-ene **2a** on treatment with formic acid/chloroform.[16] For the ursane series, the use of P_2O_5 as dehydrating agent[17] gave rise directly to the ring A-contracted alkene **2b**.

Cleavage of the double bond in the contracted A-ring of **2a** and **2b** by ruthenium tetroxide, was followed by a vapour phase retro-Michael reaction[18] affording the des-A-ketones **4a** and **4b** respectively. For the ursane series, the use of one equivalent of ruthenium tetroxide ensured little or no attack of the hindered Δ^{12} double bond.

Robinson annelation of **4b** under acidic conditions (modified from ref. 19) gave rise directly to **5b** in moderate yield. Under basic conditions, virtually no annelated product could be recovered. Annelation of **4a** under identical acidic conditions, proceeded at a much slower rate and with poorer yields ; however, since most unreacted starting material could be recovered, the reaction was repeated several times in order to obtain adequate quantities of product.

2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation of **5a** to **6a** and of **5b** to **6b** gave predominantly the $\Delta^{1,4}$-3-one as expected (cf. ref. 20 for steroidal ketones) and a small amount of another dienone, probably the $\Delta^{4,6}$-3-one.

Reduction of the mixtures with lithium aluminium hydride, to the dienols, and acidic work up[21] afforded **7a** and **7b**, respectively, which could be readily separated from other rearranged products (see Experimental). The position of the 4-methyl group on the aromatic ring of both compounds was confirmed by ^{1}H-NMR. ^{1}H selective decouplings allowed unambiguous assignment of the two benzylic protons at C-6, and a differential nuclear Overhauser effect experiment showed connectivities between the two H-6 and the benzylic methyl group, which is in agreement with the structures of **7a** and **7b**.

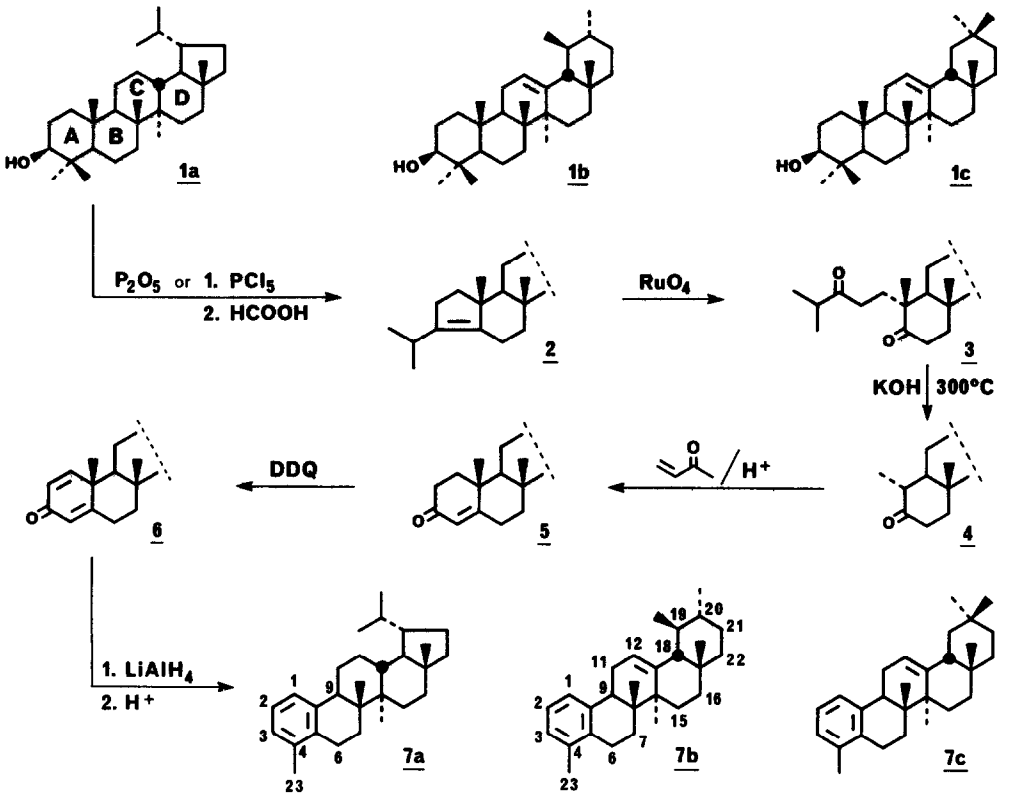

Figure 2 : Synthetic scheme for the preparation of the pentacyclic monoaromatic triterpenoids 7a-c

Synthesis of the tetracyclic monoaromatic triterpenoid 10.

The synthesis of **10** is outlined in figure 3. Hence, oxidation with DDQ of **4a**, afforded **8**, as a mixture of itself and des-*A*-lup-6-en-5-one, des-*A*-lup-9-en-5-one and des-*A*-lupa-6,9,11-trien-5-one, which could be readily separated by TLC.

Reductive aromatisation of **8** with lithium/biphenyl afforded a good yield of **9**, as indicated previously in similar reductive aromatisations of steroids.[22]

Conversion of the tetramethyldiamidophosphate ester of **9** to the aromatic hydrocarbon **10**, was carried out by a lithium/ethylamine reduction.[23]

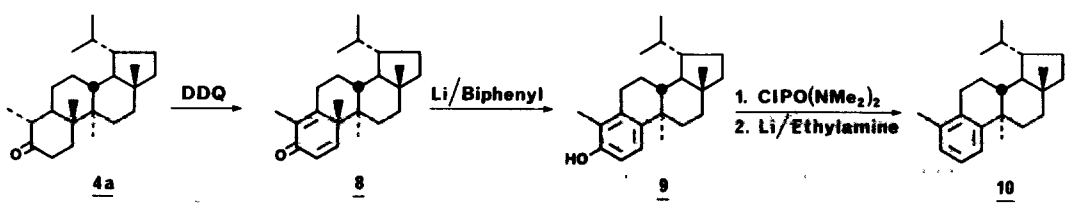

Figure 3 : Synthetic scheme for the preparation of des-*A*-26-norlupa-5,7,9-triene **10**

Occurrence and identification of higher plant derived monoaromatic triterpenoids in sediments.

The synthesis of **7a-c** and **10** has allowed their identification in sediments from the Mahakam Delta (Indonesia), by comparison of their mass spectra, and by their co-elution on polar and apolar gas chromatographic columns with their sedimentary counterparts. It should be noted that mass spectra of all the pentacyclic compounds exhibit a prominent fragmentation peak at *m/z* = 145. Figure 4 shows the distribution of the pentacyclic monoaromatic hydrocarbons **7a-c** from a core sample (463 m) where **7a** is the major compound of the fraction. **10** always occurs as a minor component in these samples, detectable only in the course of GC-MS studies. However, this latter appears as a significant product in the Eocene Messel oil shale (Germany).

Analysis of a number of other sediment and soil samples[24,25] indicates that the monoaromatic triterpenoids occur commonly, and that they are formed rapidly in the subsurface. Indeed **7a**, **10** and **7c** were identified as significant microbial degradation products of radiolabelled lupan-3-one and olean-12-en-3β-ol, after their incubation with a recent sediment, thus providing direct evidence that the aromatisation of triterpenoids in sediments proceeds *via* a microbial process (the detailed results of this work will be published elsewhere).[25,26]

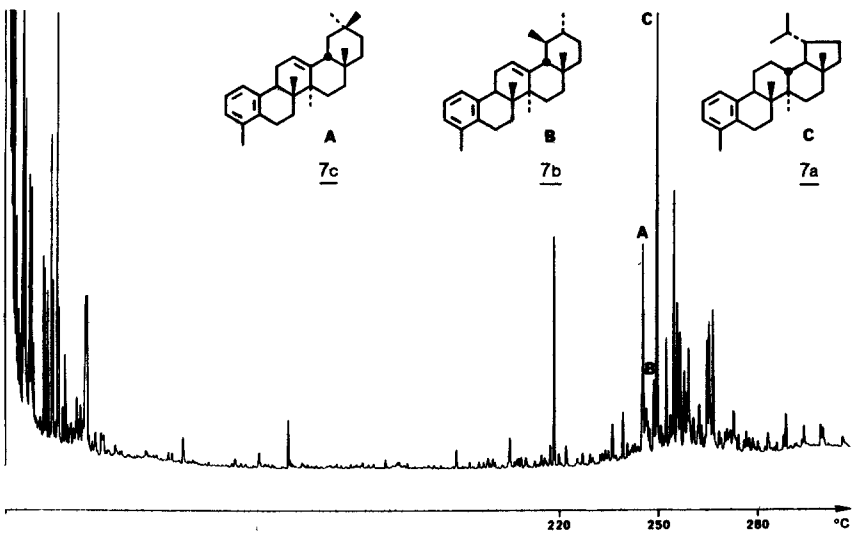

Figure 4 : Gas chromatogram of the total aromatic hydrocarbons from a core sample of the Mahakam Delta (373 m)
Conditions : SE-54, 26 m x 0.3 mm; 40-280°C, 3°C/min; carrier gas H_2 *ca* 2 ml/min

EXPERIMENTAL

General

Gas Chromatography (GC) : Analyses were carried out using a Carlo Erba Fractovap 4160 chromatograph with on-column injector and flame ionisation detector and fitted with WCOT glass columns (SE-54 phase obtained from Alltech Associates). **High Performance Liquid Chromatography (HPLC)** : A Waters 590EF pump with a Waters U6K injector were used with both semi-preparative and analytical columns. Detection was carried out with a Waters differential refractometer R401, refractive index detector. Typical flow rates for semi-preparative (Du Pont 250 x 21.2

mm, Zorbax ODS 8 µm) and analytical (Du Pont Golden series 80 x 6.2 mm, Zorbax ODS 3 µm) were 18 and 2 ml/min respectively. **Gas Chromatography - Mass Spectrometry (GC-MS)** : Analyses were performed on a Carlo Erba FTV 4160 gas chromatograph with fused silica columns 30 m x 0.25 mm, DB5 (apolar) or DB17 (polar), helium carrier gas (*ca* 2 bar), typically temperature programmed from 40-100°C at 10°C/min, 100-300°C at 4°C/min and then isothermal. The 4160 chromatograph was interfaced with a Kratos MS 80RF mass spectrometer. Typical operating conditons were : ion source 250°C, electron energy 70 eV. The mass spectrometer was interfaced with a Kratos DS55 data system. GC-MS co-injections of reference compounds with geological samples were carried out using the Kratos MS 80 in MID mode. **Electron Impact Direct Insertion Mass Spectrometry (EIMS)** : Conditions were as for GC-MS except that the sample introduction was *via* direct insertion probe. **High Resolution Mass Spectrometry (HRMS)** : High resolution spectra were obtained using the Kratos MS 80RF mass spectrometer. Samples were introduced *via* direct insertion probe. The instrument was scanned cyclically at 10 s/decade from 50-600 and operated at a resolution of 3000. Measurement was by computer peak matching with internal standard (Perfluorokerosene). **^{1}H Nuclear Magnetic Resonance (^{1}H-NMR)** : 400 MHz ^{1}H-NMR spectra were obtained on a Bruker AM 400 instrument. The chemical shifts (δ) are reported in ppm downfield from TMS and are internally referenced to the residual protons in the deuterated solvents. Coupling constants (*J*) are given in Herz. Solvent was $CDCl_3$ (δ_H 7.27 ppm) unless otherwise stated. **UV** spectra were taken on a diode array spectrophotometer Hewlett-Packard 8451.

Chemical syntheses

***A*-Neolup-3(5)-ene 2a** : Prepared from lupanol **1a**.[15,16] EIMS (GC) 70 eV, *m/z* (rel. int.) : 410(M^+, 24%), 395(17), 367(100), 231(30), 189(11), 175(19), 161(29), 137(23), 136(53), 135(45), 121(50). ^{1}H-NMR : δ 0.77(3H, *d*, *J*=6.9), 0.78(3H, *s*), 0.85(3H, *d*, *J*=6.9), 0.87(3H, *s*), 0.91(3H, *s*), 0.93(3H, *d*, *J*=6.9), 0.99(3H, *d*, *J*=6.9), 1.12(3H, *s*), 2.65(1H, septet, *J*=6.9).

4,5-Secolupane-3,5-dione 3a : Prepared from *A*-neolup-3(5)-ene **2a**.[18] EIMS (direct insertion) 70 eV, *m/z* (rel. int.) : 442(M^+,3%), 427(10), 424(16), 399(20), 344(64), 329(8), 301(22), 231(30), 206(26), 191(35), 163(43), 149(45), 123(85), 95(90), 69(100), 55(500), normalised to *m/z* 69 as 100%. ^{1}H-NMR : δ 0.78(3H, *s*), 0.78(3H, *d*, *J*=6.9), 0.86(3H, *d*, *J*=6.9), 0.97(3H, *s*), 1.04(3H, *s*), 1.08(3H, *d*, *J*=6.9), 1.09(3H, *d*, *J*=6.9), 1.12(3H, *s*), 2.07(1H, *dd*, *J*=3.6,12.5), 2.20-2.50(4H), 2.61(1H, septet, *J*=6.9).

(10βH)-Des-*A*-lupan-5-one 4a : Prepared from 4,5-secolupane-3,5-dione **3a**.[18] Mp : 171-172°C. EIMS (GC) 70 eV, *m/z* (rel. int.) · 344(M^+,27%), 329(9), 301(28), 232(8), 231(11), 218(9), 206(32), 191(31), 163(59), 138(73), 123(91), 95(94), 55(100). ^{1}H-NMR : δ 0.77(3H, *d*, *J*=6.8), 0.81(3H, *s*), 0.85(3H, *d*, *J*=6.8), 0.93(3H, *s*), 0.98(3H, *d*, *J*=6.5), 1.27(3H, *s*), 2.21-2.33(2H), 2.47(1H, *ddd*, *J*=7.0,13.8,13.8).

Des-*A*-lupa-6,9-dien-5-one 8 : Typically **4a** (100 mg) in toluene (2ml) with DDQ (200 mg) was sealed in a glass tube under vacuum after purging with argon (x 5). After heating (100°C, 12 h), the tube was opened and the content deposed on alumina (5 g) and eluted with ethyl acetate (20 ml). Thin layer chromatography (silica gel, methylene chloride) afforded pure **8** (Rf 0.2; 37 mg, 37%). HRMS M^+, m/e : 340.278; $C_{24}H_{36}O$ requires : 340.277. EIMS (GC) 70 eV, *m/z* (rel. int.) : 340(M^+,18%), 325(7), 297(12), 205(14), 149(35), 136(83), 135(100), 91(47). ^{1}H-NMR : δ 0.70(3H, *s*), 0.76(3H, *d*, *J*=6.7), 0.87(3H, *s*), 0.88(3H, *d*, *J*=6.5), 1.34(3H, *s*), 1.92(3H, *s*), 2.24(1H, *ddd*, *J*=5.5,13.8,13.8), 2.84(1H, *ddd*, *J*=2.3,4.8,14.4), 6.30(1H, *d*, *J*=10 1), 6.97(1H, *d*, *J*=10.1).

Des-*A*-26-norlupa-5,7,9-trien-5-ol 9 : To a refluxing solution of biphenyl (60 mg), diphenylmethane (30 mg) and lithium (100 mg) in dry THF (4 ml) under argon, **8** (20 mg) in THF (2 ml) was added dropwise once a green colour had developed. After further reflux (10 min) and cooling, methanol (3 ml) was added in order to destroy excess lithium. The solvent was removed and residues were dissolved in HCl (10% v/v, 5 ml) and extracted with methylene chloride (2 x 10 ml). The organic phase was washed with water (1 x 5 ml), dried (anh. sodium sulphate) and the solvent removed *in vacuo*. TLC (silica gel, methylene chloride), afforded pure **9** (Rf 0.5; 8 mg, 42%). EIMS (GC) 70 eV, *m/z* (rel. int.) : 326(M^+,66%), 311(100, saturated), 187(27), 173(97), 143(43). ^{1}H-NMR (CD_2Cl_2 δ_H 5.32) : δ 0.78(3H, *s*), 0.83(3H, *d*, *J*=6.8), 0.90(3H, *d*, *J*=6.8), 1.07(3H, *s*), 2.07(3H, *s*), 2.57-2.76(2H), 6.58(1H, *d*, *J*=8.5), 7.03(1H, *d*, *J*=8.5).

Des-*A*-26-norlupa-5,7,9-triene 10 : To a stirring solution of **9** (5 mg) in dry 1,2-dimethoxyethane (5 ml at -30°C, under argon, *n*-butyl lithium was added until in excess (triphenylmethane indicator, 1 mg, light pink colour). Tetramethyldiamidophosphochloridate[27] (200 µl) was then added dropwise, and the solution was stirred at ambient temperature (4 h). After addition of water (5 ml), extraction with ether (2 x 5 ml), and removal of the solvent *in vacuo*,

the crude product was subjected to TLC (silica gel, methylene chloride/ethylacetate, 50:50). The TMDAP ester of **9** (Rf 0.65) was isolated and dissolved in THF/*t*-butanol (50:50, 2 ml). This solution was then added dropwise to a stirring (deep blue) solution of lithium (50 mg) in ethylamine (anhydrous, 5ml, -30°C). Excess lithium was immediately destroyed with methanol (2 ml). After adding water (5 ml), the solution was extracted (chloroform, 2 x 5 ml), and the organic phase washed with water (1 x 5 ml), and dried (anh. sodium sulphate). The crude product was subjected to TLC (silica gel, hexane), allowing pure **10** to be isolated (Rf 0.6; 1.0 mg, 20% overall yield). HRMS M^+, *m/e* : 310.263; $C_{23}H_{34}$ requires : 310.266. EIMS (GC) 70 eV, *m/z* (rel. int.) : 310(M^+,22%), 295(100), 225(5), 211(5), 197(8), 183(7), 171(21), 157(87), 145(23), 143(27), 142(17), 137(12), 131(89), 95(24). ^{1}H-NMR (CD_2Cl_2 δ_H 5.32) : δ 0.78(3H, *s*), 0.83(3H, *d*, *J*=6.8), 0.91(3H, *d*, *J*=6.8), 1.10(3H, *s*), 2.18(3H, *s*), 6.94(1H, *d*, *J*=7.2), 7.01(1H, *dd*, *J*=7.2,7.9), 7.17(1H, *d*, *J*=7.9).

23,24-Dinorlup-4-en-3-one 5a : **4a** (78 mg) in benzene (15 ml) was refluxed (24 h) with *p*-toluenesulphonic acid (15 mg) and methylvinyl ketone (400 µl). The solution was filtered, washed with sodium bicarbonate solution (5% aqueous, 1 x 10 ml) and water (1 x 5 ml), dried (anh. sodium sulphate) and the solvent removed *in vacuo*. TLC of the crude product (silica gel, ethylacetate/methylene chloride, 5:95) afforded the starting material **4a** (Rf 0.75; 38 mg) and the required product **5a** (Rf 0.6), which was further purified to remove any polymeric impurities by semi-preparative HPLC (Zorbax ODS, methanol, t_R 19 min; 21 mg, 46% yield relative to transformed product). HRMS M^+, *m/e* : 396.340; $C_{28}H_{44}O$ requires : 396.339. EIMS (GC) 70 eV, *m/z* (rel. int.) : 396(M^+,28%), 381(8), 353(11), 273(10), 272(9), 231(32), 191(39), 124(100), 123(70), 95(70). ^{1}H-NMR : δ 0.77(3H, *d*, *J*=6.8), 0.79(3H, *s*), 0.86(3H, *d*, *J*=6.8), 0.92(3H, *s*), 1.17(3H, *s*), 1.25(3H, *s*), 2.06(1H, *ddd*, *J*=2.6,5.3,13.3), 2.17(1H, *ddd*, *J*=3.2,3.2,14.5), 2.33(1H, *m*), 2.46(1H, *ddd*, *J*=5.2,14.9,17.4), 2.57(1H, *m*), 5.75(1H, br *s*).

23,24-Dinorlupa-1,4-dien-3-one 6a : 23,24-Dinorlup-4-en-3-one **5a** (20 mg) with DDQ (40 mg) in toluene (2 ml) were sealed in glass tube under vacuum after purging with argon (x 5). After heating, the sample was worked up as for **8**. TLC of the crude product (silica gel, ethyl acetate/hexane, 25:75) afforded the required product **6a** (13 mg, 65%). HRMS M^+, *m/e* : 394.325; $C_{28}H_{42}O$ requires 394.324. EIMS (GC) 70 eV, *m/z* (rel. int.) : 394(M^+,19%), 379(5), 351(17), 231(24), 202(73), 187(30), 161(58), 149(39), 121(100); ^{1}H-NMR : δ 0.75(3H, *d*, *J*=6.8), 0.79(3H, *s*), 0.84(3H, *s*), 0.85(3H, *d*, *J*=6.8), 1.22(3H, *s*), 1.31(3H, *s*), 2.78(1H, *ddd*, *J*=3.0,3.0,13.3), 2.64(1H, br *ddd*, *J*=4.5,8.8,8.8), 6.10(1H, br s), 6.14(1H, *dd*, *J*=1.8,10.1), 7.06(1H, *d*, *J*=10.1). λ_{max} 246 nm (CH_2Cl_2) or 248 nm (ethanol) [cholesta-1,4-dien-3-one λ_{max} 246 nm (CH_2Cl_2)].

24,25-Dinorlupa-1,3,5(10)-triene 7a : 23,24-Dinorlupa-4,6-dien-3-one **6a** (20 mg in 1 ml diethylether) was added dropwise to a stirring solution of $LiAlH_4$ (100 mg) in diethylether (30 ml). When the addition was complete, ether (2 ml) was added and the solution refluxed (20 min). After cooling, excess $LiAlH_4$ was destroyed with ethylacetate. The resultant copious white precipitate was dissolved in HCl (5% aqueous, 10 ml), diluted with water, and the organic layer separated. The aqueous layer was extracted with ether (3 x 5 ml), and the combined organic extracts washed with water (1 x 10 ml) and dried (anh. sodium sulphate). After removal of solvent *in vacuo*, the crude product was subjected to TLC (silica gel, hexane), affording **7a** as a mixture of itself (20% by GC) and other rearranged components. Analytical scale HPLC (Zorbax ODS, MeOH, t_R 15 min) afforded pure **7a** (2 mg, 10% overall yield). HRMS M^+, *m/e* : 378.329; $C_{28}H_{42}$ requires : 378.329. EIMS (GC) 70 eV, *m/z* (rel. int.) : 378(M^+,7%), 363(<1), 335(<1), 233(3), 172(18), 158(13), 157(24), 146(32), 145(100), 144(38). ^{1}H-NMR : δ 0.80(3H, *s*), 0.80(3H, *d*, *J*=6.9), 0.87(3H, *s*), 0.88(3H, *d*, *J*=6.9), 1.01(3H, *s*), 2.22(3H, *s*, H-23), 2.25-2.33(1H, *m*), 2.60(1H, *ddd*, *J*=6,12,17, H-6β), 2.67(1H, *ddd*, *J*=3,7,17, H-6α), 2.90(1H, br *dd*, *J*=3.5,12.0, H-9), 6.99(1H, *d*, *J*=7.7, H-3), 7.07(1H, *dd*, *J*=7.7,8.2, H-2), 7.15(1H, *d*, *J*=8.2, H-1).

The synthesis of **7b** and **7c** was carried out on a mixture of their respective starting materials, urs-12-en-3β-ol **1b** and olean-12-en-3β-ol **1c** (90:10). At each stage, an aliquot of the ursane component was separated for analytical purposes by analytical reverse phase HPLC (Zorbax ODS). In the oleanane series, separation and complete characterisation was only carried out on the final product **7c**.

***A*-Neoursa-3(5),12-diene 2b** : Urs-12-en-3β-ol **1b** (1.3 g) was refluxed with toluene (200 ml) in a Dean-Stark apparatus.[17] The volume of solvent was reduced (50 ml over 2 h), and the solution cooled (0°C). Phosphorus pentoxide (*ca* 1 g) was added to the stirring solution under argon. After 2 h, the reaction was complete as monitored by TLC. The solution was poured into sodium carbonate solution (aqueous, saturated), and the layers separated. The organic layer was washed with water (1 x 50 ml), dried (anh. sodium sulphate) and the solvent removed *in vacuo*. Column chromatography (silica gel, hexane) afforded **2b** (650 mg, 52%). HRMS M^+, *m/e* : 408.377; $C_{30}H_{48}$ requires : 408.376. EIMS (GC) 70 eV, *m/z* (rel. int.) : 408(M^+,17%), 393(17), 365(100), 271(31), 272(27), 257(13), 231(18), 218(14), 203(28), 190(20), 189(18), 175(47), 161(26), 147(23), 135(32), 134(17), 133(24), 121(40). ^{1}H-NMR : δ 0.80(3H, br *d*, *J*=6.0),

0.83(3H, *s*), 0.92-0.94(3H, distorted *d*), 0.94(3H, *d*, *J*=6.8), 0.97(3H, *s*), 1.00(3H, *d*, *J*=6.8), 1.04(3H, *s*), 1.11(3H, *s*), 2.67(1H, septet, *J*=6.8), 5.21(1H, *dd*, *J*=2.5,4.5).

4,5-Secours-12-ene-3,5-dione 3b : To **2b** (650 mg) in carbon tetrachloride (50 ml) was added dropwise a solution of ruthenium tetroxide in the same solvent. The addition was continued, until no starting material remained, as monitored by TLC. Filtration through celite to remove the ruthenium dioxide precipitate, removal of the solvent *in vacuo* and column chromatography (silica gel) with ethyl acetate/hexane (20:80) as eluate gave **3b** (500 mg, 72%). HRMS M^+, m/e : 440.367; $C_{30}H_{48}O2$ requires : 440.365. EIMS (GC) 70 eV, *m/z* (rel. int.) : 440(M^+,23%), 425(3), 423(5), 407(6), 355(3), 342(10), 327(8), 313(6), 270(62), 257(21), 255(26), 218(11), 203(11), 183(14), 147(16), 133(36), 125(40), 119(78). ^{1}H-NMR : δ 0.80(3H, br *d*, *J*=5.9), 0.82(3H, *s*), 0.92-0.94(3H, distorted *d*), 1.08(3H, *d*, *J*=6 9), 1.09(3H, *d*, *J*=6.9), 1.11(3H, s), 1.13(6H, *s*), 2.61(1H, septet, *J*= 6.9), 5.26(1H, *dd*, *J*=2.5,4.7).

(10βH)-Des-*A*-urs-12-en-5-one 4b : Prepared from **3b**.[18] HRMS M^+, *m/e* : 342.294; $C_{24}H_{38}O$ requires : 342.292. EIMS (GC) 70 eV, *m/z* (rel. int.) : 342(M^+,53%), 327(100), 259(29), 243(10), 231(27), 218(79), 205(19), 203(40), 189(37), 163(18), 161(22), 149(20), 147(28), 138(37), 137(19), 136(29), 135'45), 134(25), 133(55), 123(31), 122(32), 121(35), 119(52), 109(45), 107(34), 105(37), 95(59). ^{1}H-NMR : δ 0.80(3H, *d*, *J*=6.0), 0.86(3H, *s*), 0.91-0.95(3H, distorted *d*), 1.02(3H, *d*, *J*=6.4), 1.06(3H, *s*), 1.26(3H, *s*), 2.20-2.33(2H), 2.50(1H, *ddd*, *J*=5.9,13.9,13.9), 5.21(1H, *dd*, *J*=2.5,4.5).

23,24-Dinorursa-4,12-dien-3-one 5b : **4b** (150 mg) in toluene (15 ml) was refluxed (6 h) with *p*-toluenesulphonic acid (50 mg) and methylvinyl ketone (500 µl). On cooling the solution was filtered through an alumina plug (20 g), which was then washed with ethyl acetate (50 ml). After removal of the solvent *in vacuo*, TLC of the crude product (silica gel, ethyl acetate/methylene chloride, 5:95) afforded the starting material **4b** (Rf 0.75, 60 mg) and **5b** (Rf 0.6). **5b** was further purified to remove any polymeric impurities by semi-preparative HPLC (Zorbax ODS, methanol, t_R 18 min; 45 mg, 50% yield of converted product). HRMS M^+, *m/e* : 394.325; $C_{28}H_{42}O$ requires : 394.324. EIMS (GC) 70 eV, *m/z* (rel. int.) : 394(M^+,15%), 379(10), 271(12), 270(14), 232(22), 231(20), 218(100), 203(44), 189(28), 177(35), 163(86), 162(71), 147(38), 135(49), 133(72), 119(72). ^{1}H-NMR : δ 0.80(3H, *d*, *J*=6.0), 0.84(3H, *s*), 0.91-0.95(3H, distorted *d*), 1.06(3H, *s*), 1.23(3H, *s*), 1.28(3H, *s*), 2.19(1H, *ddd*, *J*=3.5,3.5,14.0), 2.33(1H), 2.42-2.57(2H), 5.23(1H, *dd*, *J*=2.7,4.6), 5.77(1H, br *s*).

23,24-Dinorursa-4,6,12-trien-3-one 6b : **5b** (45 mg) with DDQ (110 mg) in toluene (2 ml) were sealed in a glass tube, after purging with argon (x 5). After heating (100°C, 6 h), the sample was worked up as for **8**. TLC of the crude product (silica gel, ethyl acetate/hexane, 25:75) afforded **6b** as the major product (30 mg, 66%). EIMS (GC) 70 eV, *m/e* (rel. int.) : 392(M^+,58%), 377(8), 271(30), 268(18), 257(23), 231(23), 229(29), 203(19), 175(27), 161(64), 147(100), 133(48), 121(75).

24,25-Dinorursa-1,3,5(10),12-tetraene 7b : **6b** (18 mg in 1 ml diethylether) was added dropwise to a stirring solution of $LiAlH_4$ (100 mg) in diethylether (30 ml). When the addition was complete, ether (2 ml) was added and the solution refluxed. After cooling, excess $LiAlH_4$ was destroyed with ethyl acetate. The resultant copious white precipitate was dissolved in HCl (5% aqueous, 10 ml), diluted with water, and the organic layer separated. The aqueous layer was extracted with ether (3 x 5 ml), and the combined organic extracts washed with water (1 x 10 ml) and dried (anh. sodium sulphate). After removal of solvent *in vacuo* the crude product was subjected to TLC (silica gel, hexane) affording **7b** as a mixture of itself (30%) and other rearranged components. Analytical scale HPLC (Zorbax ODS, methanol, t_R 13 min) gave rise to pure **7b** (3.5 mg, 14% overall yield). HRMS M^+, *m/e* : 376.314; $C_{28}H_{40}$ requires . 376.313. EIMS (GC) 70 eV, *m/z* (rel; int.) : 376(M^+,9%), 361(8), 218(6), 204(2), 203(3), 172(8), 171(9), 170(6), 169(6), 158(94), 157(13), 145(100), 143(29), 131(21), 105(16). ^{1}H-NMR : δ 0.86(3H, *s*), 0.84-0.88(3H), 0.92(3H, *s*), 0.93-0.97(3H, distorted *d*), 1.15(3H, *s*, H-27), 1.66(1H, *ddd*, *J*≈2,5,13, H-7β), 1.75(1H, *ddd*, *J*≈6,12,13, H-7α), 1.89-2 00(2H, H-11β and H-15β), 2.09(1H, *ddd*, *J*=4.2,13.3,13 3, H-16α), 2 25(3H, *s*, H-23), 2.57(1H, *ddd*, *J*≈5,12,17, H-6β), 2.69(1H, *ddd*, *J*≈2,6,17, H-6α), 2.73(1H, *ddd*, *J*≈4 5,6,18, H-11α), 3 11(1H, br *dd*, *J*≈6,11, H-9), 5 35(1H, *dd*, *J*=2.5,4 5, H-12), 7.00(1H, *d*, *J*=7.2, H-3), 7.10(1H, *dd*, *J*=7.2,7.9, H-2), 7 15(1H, *d*, *J*=7 9, H-1).

24,25-Dinoroleana-1,3,5(10),12-tetraene 7c : separated from **7b** as a by-product by analytical scale HPLC (Zorbax ODS, methanol, t_R 11.5 min). HRMS M^+, *m/e* : 376.312; $C_{28}H_{40}$ requires 376.313. EIMS (GC) 70 eV, *m/z* (rel. int) : 376(M^+,13%), 361(13), 218(4), 204(8), 189(6), 172(10), 171(6), 170(5), 158(100), 157(11), 145(94), 143(20), 131(13), 105(9). ^{1}H-NMR : δ 0.89(3H, *s*), 0.90(3H, *s*), 0.91(6H, 2 x *s*), 1.22(3H, *s*), 1.66(1H, *ddd*, *J*≈3,6,13, H-7β), 1.77(1H, *ddd*, *J*≈6,12,13, H-7α), 1.78(1H, *dd*, *J*≈13,13, H-19α), 1 88(1H, *ddd*, *J*≈4,12,12, H-15β), 1.94(1H, *ddd*, *J*≈2.5,11,17.5, H-11β), 2.06(1H, *dd*, *J*≈4,13, H-18), 2.08(1H, *ddd*, *J*=4.2,13 9,13.9, H-16α), 2.24(3H, *s*, H-23),

2.57(1H, *ddd*, *J*=6,12,17, H-6β), 2.63-2.73(2H, H-6α and H-11α), 3.13(1H, *dd*,*J*=6,11, H-9), 5.42(1H, *dd*, *J*=2.5,4.0, H-12), 7.00(1H, *d*, *J*=7.1, H-3), 7.09(1H, *dd*, *J*=7.1,8.0, H-2), 7.13(1H, *d*, *J*=8.0, H-1).

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