PREPARATION OF 2,3-SECODIACIDS OF THE LUPANE SERIES*

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2,3-Seco-2,3-diacids of the 28-hydroxy-20(29)-lupene and 28-hydroxylupane series (*VIIa* and *VIIb*, respectively) and their derivatives *VIII – XIV* were prepared from 28-acetoxy-20(29)-lupen-3-one (*IIIa*) and 28-acetoxy-3-lupanone (*IIIb*), respectively, by oxidation to diosphenols *Va* and *Vb* and subsequent oxidative cleavage of the C(2)–C(3) bond.

Triterpenoid A-seco derivatives are considerably widespread in nature and are assumed to be a part of the plant defense system[1]. Most of the natural A-seco compounds are 3,4-seco-3-acids and their derivatives; they have been studied also from the biological viewpoint (e.g. their antibacterial activities; see refs[1,2] and references therein). On the other hand, 2,3-secotriterpenoids are less common; some of them were also isolated from natural sources (see, e.g. refs[1,3–6]), others were obtained by oxidative cleavage of known triterpenoids[7–9], including lupane derivatives[10,11]. Oxidative cleavage of ring A is generally considered[1] to be a simple way of introducing biological activity into triterpenoid derivatives, comparable with other types of oxidative functionalization.

In the present paper we describe the preparation of 2,3-seco-2,3-dioic acids of the lupane series with hydroxy group in position 28. Two parallel series of the acids were synthesized: unsaturated acids (series *a*), prepared from 20(29)-lupene-3β,28-diol (*Ia*) as the starting compound, and saturated acids (series *b*) the synthesis of which started from the dihydro derivative of *Ia*, 3β,28-lupanediol (*Ib*). Both diols *Ia* and *Ib* were acetylated with acetic anhydride in pyridine at 0 °C (for the procedure see ref.[12]) to give 28-monoacetates *IIa* (described in ref.[12,13]) and *IIb* (ref.[14]) which were oxidized with potassium dichromate to ketones *IIIa* (ref.[13]) and *IIIb*. Alkaline hydrolysis of the

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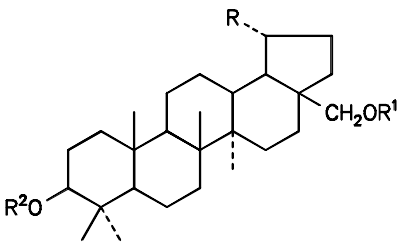
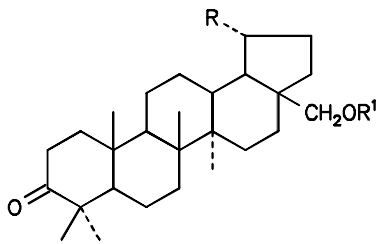
acetate groups led to hydroxy ketones *IVa* (ref.[15]) and *IVb* (ref.[16]). The ketone *IVa* was recently isolated[17] from the bark of *Betula lenta* L.

Ketones *IIIa* and *IIIb* were oxidized with oxygen in the presence of potassium *tert*-butoxide to give diosphenols *Va* and *Vb*. With triterpenoid derivatives, this reaction is used (see e.g. refs[10,18–20]) with small variations in the experimental conditions and gives good yields of the desired 2,3-diketones. In our case, the best results were obtained when the oxidation was performed in *tert*-butyl alcohol at about 40 °C for 20 – 30 min, the yields of diosphenols *Va* and *Vb* being 70 – 80%. The compounds *Va* and *Vb* were accompanied with only minor amounts of further oxidative degradation product which, according to spectral data and analogy[19], are lactols *VIa* and *VIb*.



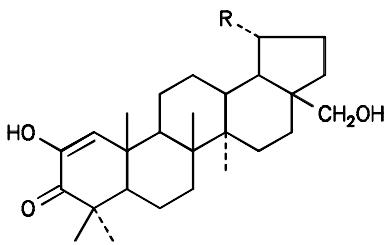


I, $R^1 = R^2 = H$

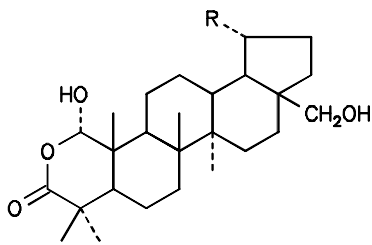
II, $R^1 = COCH_3$; $R^2 = H$

III, $R^1 = COCH_3$

IV, $R^1 = H$



V



VI

In formulae *I* – *VI* : *a*, R = $C(CH_3)=CH_2$; *b*, $CH(CH_3)_2$

By analogy[7,10], diosphenols *Va* and *Vb* were oxidatively cleaved with hydrogen peroxide in methanol in the presence of potassium hydroxide. As the principal products we obtained diacids *VIIa* and *VIIb*; as side-products we isolated only monomethyl esters with the ester functionality in position 2 (*IXa* and *IXb*) or 3 (*Xa* and *Xb*). These monomethyl esters, as well as the free acids *VIIa* and *VIIb*, were converted into dimethyl esters *VIIIa* and *VIIIb* by treatment with ethereal diazomethane. Partial esterification of acid *VIIa* with methanol afforded monoester *IXa*. Acetylation of dimethyl esters *VIIIa* and *VIIIb* with acetic anhydride in pyridine at room temperature afforded 28-acetates *XIa* and *XIb*; under these conditions, acids *VIIa* and *VIIb* were converted into anhydrides *XIVa* and *XIVb*.

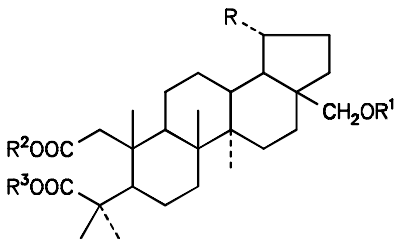
In the saturated series *b*, we tried to obtain the 2,3-seco derivatives also by oxidative cleavage of 2α-hydroxy-3-ketone *XV* which was prepared by reaction of ketone *IIIb* with *m*-chloroperoxybenzoic acid in the presence of methanol and trace of sulfuric acid according to a described[21] procedure. The α-configuration of the hydroxy group on C(2) in ketone *XV* follows from the values[22] of coupling constants $J(1\alpha,2\beta)$ (12.7 Hz) and $J(1\beta,2\beta)$ (6.8 Hz). Oxidation of the hydroxy ketone *XV* with lead tetraacetate in acetic acid at room temperature led to complex mixtures of products among which we identified only anhydride *XIVb*. More advantageous was oxidation in acetic acid in the presence of methanol: this gave the anhydride *XIVb* (24%) together with a mixture of monomethyl esters *XII* and *XIII* (55%) which were not separated. According to ^{1}H NMR spectrum, compound *XII* predominated in the mixture in the ratio 2 : 1. On treatment with diazomethane, the mixture of monomethyl esters was converted into dimethyl ester *XIb*.

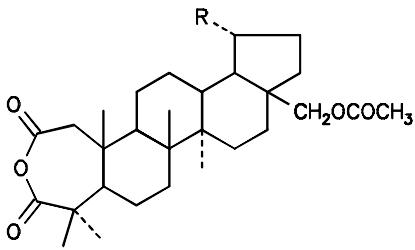
The structure of the prepared compounds was confirmed by infrared, ^{1}H NMR and mass spectra. In the mass spectra of 2,3-secodiacids *VIIa* and *VIIb* and their esters *VIIIa* and *VIIIb* – *XIa* and *XIb*, cleavage of the C(1)–C(10) and C(4)–C(5) bonds represented the main fragmentation trend. The former cleavage leads to elimination of radical $ROOC(2)–C(1)H_2^\bullet$ (59 and 73 mass units for R = H and R = CH_3, respectively) or, with hydrogen transfer, to elimination of acetic acid or its methyl ester (loss of 60 or 74 mass units). Analogously, cleavage of the C(4)–C(5) bond results in loss of radical $[ROOC(3)–C(4)(CH_3)_2]^\bullet$ (87 or 101 units for R = H or CH_3) or a molecule of 2-methylpropanoic acid or its methyl ester (88 or 102 mass units). Moreover, the molecule loses the substituent in position 17β (loss of radical $\bullet CH_2OR$, where R = H or $COCH_3$) or a molecule of water or acetic acid. The spectra exhibit relatively abundant ions corresponding to combination of the mentioned losses; in the low mass region there are ions of *m*/*z* 88 or 102, corresponding to 2-methylpropanoic acid or its methyl ester. On the basis of this fragmentation it was possible to determine position of the ester and free carboxyl groups in the monoesters *IXa*, *IXb*, *Xa* and *Xb*.

The isomeric monomethyl esters can also be distinguished by IR and ^{1}H NMR spectra: in the IR spectra of 2-methyl esters *IXa* and *IXb* both carbonyl bands appear at

lower wavenumbers (about 1 728 and 1 698 cm^{-1}) than those of *Xa* and *Xb* with the ester grouping in position 3 (1 740 and 1 718 cm^{-1}). In the ^1H NMR spectra, the chemical shifts of methoxyl singlets in 2-methyl esters *IXa* and *IXb* (δ 3.62) differ from those in 3-methyl esters *Xa* and *Xb* (δ 3.67).

Compounds *VIa*, *VIIa*, *VIIb*, *IXa*, *IXb*, *Xa* and *Xb* exhibited no antibacterial activity against *Escherichia coli*.





XIV

VII, $R^1 = R^2 = R^3 = H$

VIII, $R^1 = H$; $R^2 = R^3 = CH_3$

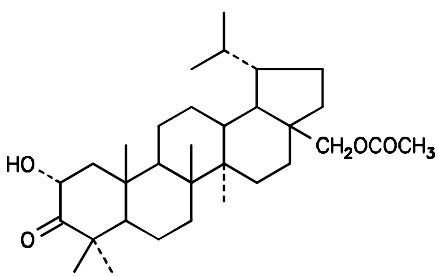
IX, $R^1 = R^3 = H$; $R^2 = CH_3$

X, $R^1 = R^2 = H$; $R^3 = CH_3$

XI, $R^1 = COCH_3$; $R^2 = R^3 = CH_3$

XII, $R = CH(CH_3)_2$; $R^1 = COCH_3$; $R^2 = CH_3$; $R^3 = H$

XIII, $R = CH(CH_3)_2$; $R^1 = COCH_3$; $R^2 = H$; $R^3 = CH_3$



XV

In formulae *VII* – *XI*; *XIV* : *a*, $R = C(CH_3)=CH_2$; *b*, $CH(CH_3)_2$

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. The optical rotations were measured in chloroform (*c* 0.3 – 0.8) on an automatic polarimeter ETL-NPL (Bendix–Ericsson), accuracy ±2°. Infrared spectra were recorded in chlorofom on a PE 684 (Perkin–Elmer) spectrometer, wavenumbers are given in cm^{-1}. The ^1H NMR spectra were measured on an FT-NMR spectrometer Tesla BS 587 A (80 MHz) in deuteriochloroform with tetramethylsilane as internal standard. The values of proton chemical shifts (ppm, δ-scale) and interproton coupling constants (in Hz) were obtained by the first order analysis; the two-spin systems were analyzed as AB systems. Mass spectra were measured on an INCOS 50 (Finnigan MAT) spectrometer, ionizing electron energy 70 eV, ion source temperature 150 °C. The samples were introduced from direct exposure probe at heating rate 10 mA/s.

The identity of substances prepared by different procedures was checked by thin-layer chromatography, melting points and IR spectra. Thin-layer chromatography (TLC) was carried out on silica gel G (Merck), detection by spraying with 10% sulfuric acid and heating, or on Silufol foils (Kavalier, Votice), detection by 5% ethanolic phosphomolybdic acid and heating. Preparative TLC was performed on silica gel G (Merck), column chromatography on silica gel Silpearl (Kavalier, Votice). The "usual work-up procedure" denotes partition of the reaction mixture between water and ether, washing the ethereal phase successively with water, dilute hydrochloric acid or sodium hydrogen carbonate solution, and water, drying over sodium sulfate and evaporation of the solvent. Analytical samples were dried over phosphorus pentoxide at 100 °C under reduced pressure.

20(29)-Lupene-3β,28-diol 28-Acetate (*IIa*)

Diol *Ia* (13.9 g, 31.4 mmol) was treated with acetic anhydride (45 ml) in pyridine (100 ml) at 0 °C for 50 min according to ref.[12]. The obtained mixture was chromatographed on silica gel in benzene–ether (10 : 1 to 1: 1) to give monoacetate *IIa* (9.7 g, 64%), m.p. 217 – 219 °C (chloroform–methanol), $[\alpha]_D$ +13°. Reported[12] m.p. 216 – 218 °C, $[\alpha]_D$ +14.6°; ref.[13] reports m.p. 275 °C (ethanol–ether), $[\alpha]_D$ +11°. In addition, the mixture afforded 20(29)-lupene-3β,28-diol diacetate (2.3 g, 14%) and the starting diol *Ia* (0.5 g, 4%).

3β,28-Lupanediol 28-Acetate (*IIb*)

Diol *Ib* (8.3 g, 18.7 mmol) was converted into the title compound *IIb* (6.02 g, 66%) as described in the preceding experiment; m.p. 242 – 244 °C (chloroform–methanol), $[\alpha]_D$ −18° (reported[14] m.p. 243 – 245 °C and (with erroneous sign) $[\alpha]_D$ +21°). In addition, the mixture afforded lupane-3β,28-diol diacetate (0.80 g, 8%) and the starting diol *Ib* (0.35 g, 4%).

28-Acetoxy-20(29)-lupen-3-one (*IIIa*)

A solution of monoacetate *IIa* (5.0 g, 10.3 mmol) and potassium dichromate (2.5 g, 8.5 mmol) in acetic acid (350 ml) was set aside at room temperature for 2 days and the mixture was then worked up in the usual manner. Crystallization from methanol afforded ketone *IIIa* (4.0 g, 80%), m.p. 114 – 118 °C, $[\alpha]_D$ +36.5° (reported[13] m.p. 238 °C (ethanol–ethyl acetate), $[\alpha]_D$ +31.4}. IR spectrum: 1 725, 1 699, 1 640, 1 244, 890. Mass spectrum, *m*/*z* (%): 482 (M^+, 15), 467 (2), 422 (15), 409 (17), 379 (4), 245 (14), 203 (62), 189 (46), 43 (100).

28-Acetoxy-3-lupanone (*IIIb*)

A solution of monoacetate *IIb* (0.85 g, 1.7 mmol) and potassium dichromate (0.65 g, 2.2 mmol) in acetic acid (80 ml) was heated at 60 °C for 5 h and then set aside at room temperature for 3 days.

The mixture was worked up in the usual manner and the product was crystallized from methanol; yield 0.83 g (98%) of ketone *IIIb*, m.p. 127 – 130 °C, $[\alpha]_D$ +4°. IR spectrum: 1 725, 1 699, 1 245. ^{1}H NMR spectrum: 0.78 d, 3 H (J = 6.6); 0.84 d, 3 H (J = 6.6); 0.94 bs, 3 H; 0.96 s, 3 H; 1.03 s, 3 H and 1.08 s, 6 H (7 × CH_3); 2.05 s, 3 H (OAc); 2.3 – 2.6 m, 2 H (2 × H-2); 3.82 d, 1 H and 4.26 d, 1 H (2 × H-28, J = 11). Mass spectrum, *m*/*z* (%): 484 (M^+, 18), 441 (2), 424 (7), 411 (36), 381 (5), 205 (45), 191 (53), 43 (100). For $C_{32}H_{52}O_3$ (484.8) calculated: 79.29% C, 10.81% H; found: 79.05% C, 10.96% H.

28-Hydroxy-20(29)-lupen-3-one (*IVa*)

A solution of ketone *IIIa* (500 mg, 1.04 mmol) in benzene (5 ml) was mixed with 3% ethanolic solution (5 ml) of potassium hydroxide. After refluxing for 1 h, the mixture was processed in the usual manner to give ketone *IVa* (430 mg, 94%), m.p. 180 – 182 °C (ether–heptane), $[\alpha]_D$ +49° (reported[15] m.p. 188 – 189 °C, $[\alpha]_D$ +54°). IR spectrum: 3 624, 1 697, 1 639, 889. ^{1}H NMR spectrum: 0.93 s, 3 H; 0.99 s, 3 H; 1.02 s, 3 H; 1.07 s, 6 H and 1.75 bs, 3 H (6 × CH_3); 2.3 – 2.6 m, 2 H (2 × H-2); 3.33 d, 1 H and 3.81 bd, 1 H (2 × H-28, $J \approx 11$); 4.58 bs, 1 H and 4.69 bs, 1 H (2 × H-29). Mass spectrum, *m*/*z* (%): 440 (M^+, 25), 422 (12), 409 (65), 203 (77), 189 (69), 81 (99), 55 (100).

28-Hydroxy-3-lupanone (*IVb*)

Ketone *IIIb* (500 mg, 1.03 mmol) was hydrolyzed analogously as described for ketone *IIIa*. The title ketone *IVb* (420 mg, 92%) melted at 209 – 212 °C (ether–heptane), $[\alpha]_D$ +12° (reported[16] m.p. 210 °C). IR spectrum: 3 624, 1 698. ^{1}H NMR spectrum: 0.77 d, 3 H (J = 6.6); 0.85 d, 3 H (J = 6.4); 0.94 s, 3 H; 0.97 s, 3 H; 1.03 s, 3 H and 1.07 s, 6 H (7 × CH_3); 2.3 – 2.6 m, 2 H (2 × H-2); 3.30 d, 1 H and 3.78 d, 1 H (2 × H-28, J = 11). Mass spectrum, *m*/*z* (%): 442 (M^+, 12), 424 (2), 411 (100), 205 (55), 191 (67).

Oxidation of Ketone *IIIa*

Ketone *IIIa* (500 mg, 1.04 mmol) was added to a solution of potassium *tert*-butoxide, prepared by dissolving 1.6 g of potassium in 40 ml of *tert*-butyl alcohol. The solution was warmed to 40 °C for 30 min under constant introduction of oxygen, then poured into dilute hydrochloric acid and worked up in the usual manner. Chromatography of the product mixture (420 mg) on silica gel (40 g) in benzene and then in benzene–ether (10 : 1) afforded diosphenol *Va* (370 mg, 78%), m.p. 215 – 218 °C (ether) or 146 – 150 °C (chloroform–heptane), $[\alpha]$ +61°. IR spectrum: 3 623, 3 450, 1 666, 1 644, 889. ^{1}H NMR spectrum: 0.97 s, 3 H; 1.09 s, 3 H; 1.10 s, 3 H; 1.12 s, 3 H; 1.19 s, 3 H and 1.68 bs, 3 H (6 × CH_3); 3.34 d, 1 H (J = 11) and 3.80 dd, 1 H (J = 11 and 1.7, 2 × H-28); 4.60 m, 1 H and 4.69 m, 1 H (2 × H-29); 6.42 s, 1 H (H-1). Mass spectrum, *m*/*z* (%): 454 (M^+, 28), 436 (5), 424 (13), 423 (8), 326 (12), 229 (23), 189 (41), 43 (100). For $C_{30}H_{46}O_3$ (454.7) calculated: 79.25% C, 10.20% H; found: 79.43% C, 10.31% H.

Further elution afforded lactol *VIa* (30 mg, 6%), m.p. 194 – 196 °C (chloroform–heptane), $[\alpha]_D$ +18.5°. IR spectrum: 3 627, 3 599, 1 720, 1 640, 889. ^{1}H NMR spectrum: 1.01 s, 3 H; 1.03 s, 3 H; 1.07 s, 3 H; 1.19 s, 3 H; 1.26 s, 3 H and 1.67 bs, 3 H (6 × CH_3); 3.35 d, 1 H and 3.79 d, 1 H (2 × H-28, J = 11); 4.58 m, 1 H and 4.68 m, 1 H (2 × H-29); 5.30 bs, 1 H (H-1, $W_{1/2}$ = 15 Hz). Mass spectrum, *m*/*z* (%): 458 (M^+, 3), 440 (4), 427 (15), 412 (29), 399 (25), 245 (61), 189 (62), 95 (100). For $C_{29}H_{46}O_4$ (458.7) calculated: 75.94% C, 10.11% H; found: 75.87% C, 10.03% H.

Oxidation of Ketone *IIIb*

Ketone *IIIb* (900 mg, 1.86 mmol) was treated as described in the preceding experiment to give diosphenol *Vb* (690 mg, 81%), m.p. 219 – 222 °C (ether–heptane), $[\alpha]_D$ +13°. IR spectrum: 3 624, 3 452, 1 667, 1 645. ^{1}H NMR spectrum: 0.78 d, 3 H (J = 6.5); 0.90 d, 3 H (J = 6.5); 0.95 s, 3 H; 1.10 s, 3 H; 1.11 s, 3 H; 1.13 s, 3 H and 1.21 s, 3 H (7 × CH_3); 3.31 d, 1 H (J = 11) and 3.77 dd, 1 H (J = 11 and 1.4, 2 × H-28); 6.43 s, 1 H (H-1). Mass spectrum, *m*/*z* (%): 456 (M^+, 35), 438 (12), 426 (32), 425 (28), 328 (22), 229 (38), 191 (82), 43 (100). For $C_{30}H_{48}O_3$ (456.7) calculated: 78.90% C, 10.59% H; found: 79.03% C, 10.87% H.

Lactol *VIb* (120 mg, 14%) was obtained as a side-product; m.p. 186 – 189 °C (ether–heptane), $[\alpha]_D$ −17°. IR spectrum: 3 625, 3 602, 1 721. ^{1}H NMR spectrum: 0.76 d, 3 H (J = 6.5); 0.84 d, 3 H (J = 6.5); 1.02 s, 6 H; 1.08 s, 3 H; 1.19 s, 3 H and 1.26 s, 3 H (7 × CH_3); 3.32 d, 1 H and 3.77 d, 1 H (2 × H-28, J = 11); 5.29 bs, 1 H (H-1, $W_{1/2}$ = 16 Hz). Mass spectrum, *m*/*z* (%): 442 (M^+ – 18, 2), 429 (8), 414 (22), 411 (13), 401 (32), 247 (58), 191 (100). For $C_{29}H_{48}O_4$ (460.7) calculated: 75.61% C, 10.50% H; found: 75.55% C, 10.73% H.

Oxidative Cleavage of Diosphenol *Va*

Diosphenol *Va* (720 mg, 1.58 mmol) and 30% aqueous solution of hydrogen peroxide (2.5 ml) were added to a solution of potassium hydroxide (3 g) in methanol (110 ml). A further amount (10 ml) of hydrogen peroxide was added portionwise into the reaction mixture in the course of the 100 minutes' boiling. The reaction was quenched by pouring the mixture into dilute hydrochloric acid. The precipitate was collected on filter, dissolved in chloroform and the solution was filtered through a layer of silica gel. Chromatography on silica gel (80 g) in benzene and then benzene–ether (10 : 1 to 1 : 1) afforded successively monomethyl esters *Xa* (60 mg, 8%) and *IXa* (30 mg, 4%) and diacid *VIIa* (570 mg, 74%).

Diacid VIIa: m.p. 174 – 178 °C (ether–heptane), $[\alpha]_D$ +57°. IR spectrum: 3 621, 3 500 – 2 600, 1 706, 1 641, 891. Mass spectrum, *m*/*z* (%): 488 (M^+, 1), 470 (2.5), 457 (6), 456 (4), 439 (3), 428 (10), 401 (22), 383 (35), 371 (18), 341 (20), 189 (63), 155 (35), 121 (90), 88 (27), 95 (100).

Dimethyl ester VIIIa was prepared from diacid *VIIa* and from monoesters *IXa* and *Xa* by treatment with ethereal solution of diazomethane; m.p. 145 – 147 °C (heptane), $[\alpha]_D$ +16°. IR spectrum: 3 625, 1 731, 1 719, 1 639, 1 434, 1 154, 888. ^{1}H NMR spectrum: 0.90 s, 3 H; 1.01 s, 3 H; 1.02 s, 3 H; 1.23 s, 6 H and 1.67 bs, 3 H (6 × CH_3); 2.34 m, 4 H; 3.33 d, 1 H and 3.78 d, 1 H (2 × H-28, J = 11); 3.60 s, 3 H and 3.64 s, 3 H (2 × OCH_3); 4.57 m, 1 H and 4.66 m, 1 H (2 × H-29). Mass spectrum, *m*/*z* (%): 516 (M^+, 1), 498 (1), 486 (2), 485 (4), 457 (4), 443 (13), 442 (10), 425 (6), 415 (11), 397 (18), 385 (16), 341 (75), 169 (60), 121 (84), 102 (100), 95 (96). For $C_{32}H_{52}O_5$ (516.8) calculated: 74.38% C, 10.14% H; found: 74.12% C, 9.92% H.

Monomethyl ester IXa: m.p. 133 – 136 °C (ether–heptane), $[\alpha]_D$ +22°. IR spectrum: 3 624, 3 507 (broad), 1 728, 1 698, 1 639, 1 435, 1 157, 889. ^{1}H NMR spectrum: 0.93 s, 3 H; 1.02 s, 3 H; 1.03 s, 3 H; 1.26 s, 6 H and 1.67 bs, 3 H (6 × CH_3); 2.45 m, 4 H; 3.34 d, 1 H and 3.77 d, 1 H (2 × H-28, J = 11); 3.62 s, 3 H (OCH_3); 4.58 m, 1 H and 4.67 m, 1 H (2 × H-29). Mass spectrum, *m*/*z* (%): 502 (M^+, 0.5), 484 (0.5), 471 (1.5), 429 (2.5), 428 (2.5), 415 (4), 397 (6), 385 (5), 341 (13), 169 (15), 88 (18), 43 (100).

Monomethyl ester Xa: m.p. 248 – 250 °C (chloroform–heptane), $[\alpha]_D$ +18°. IR spectrum: 3 618, 3 507 (broad), 1 740, 1718, 1 644, 1 433, 1 148, 888. ^{1}H NMR spectrum: 0.93 s, 3 H; 1.00 s, 3 H; 1.04 s, 3 H; 1.23 s, 3 H; 1.24 s, 3 H and 1.67 bs, 3 H (6 × CH_3); 3.35 d, 1 H and 3.80 d, 1 H (2 × H-28, J = 11); 3.67 s, 3 H (OCH_3); 4.58 m, 1 H and 4.68 m, 1 H (2 × H-29). Mass spectrum, *m*/*z* (%): 502 (M^+, 1), 484 (2), 471 (3), 443 (5), 442 (6), 401 (8), 383 (16), 371 (7), 341 (9), 155 (25), 102 (100).

Acetyl derivative XIa was prepared from dimethyl ester *VIIIa* (130 mg, 0.25 mmol) by treatment with acetic anhydride (1 ml) in pyridine (5 ml) at room temperature for 3 days, followed by the usual work-up; yield 80 mg (57%) of acetyl derivative *XIa*, m.p. 134 – 136 °C, $[\alpha]_D$ +9°. IR spectrum: 1 725, 1 640, 1 434, 1 244, 1 153, 888. ^{1}H NMR spectrum: 0.90 s, 3 H; 1.00 s, 3 H; 1.03 s, 3 H; 1.23 s, 6 H and 1.67 bs, 3 H (6 × CH_3); 2.06 s, 3 H (OAc); 2.35 m, 3 H; 3.61 s, 3 H and 3.64 s, 3 H (2 × OCH_3); 3.85 d, 1 H and 4.24 bd, 1 H (2 × H-28, J = 11); 4.58 m, 1 H and 4.68 m, 1 H (2 × H-29). Mass spectrum, *m*/*z* (%): 558 (M^+, 0.5), 527 (1), 499 (2), 498 (1), 485 (6), 484 (4), 457 (3), 425 (8), 397 (16), 383 (26), 323 (26), 169 (100), 102 (53). For $C_{34}H_{54}O_6$ (558.8) calculated: 73.08% C, 9.74% H; found: 72.82% C, 10.05% H.

Anhydride *XIVa*

A solution of diacid *VIIa* (200 mg, 0.41 mmol) in a mixture of pyridine (4 ml) and acetic anhydride (1 ml) was allowed to stand at room temperature for 4 h. The usual work-up afforded anhydride *XIVa* (110 mg, 52%), m.p. 155 – 158 °C (ether–heptane), $[\alpha]_D$ +51°. IR spectrum: 1 796, 1 752, 1 731, 1 640, 1 242, 891. ^{1}H NMR spectrum: 0.99 s, 3 H; 1.04 s, 3 H; 1.07 s, 3 H; 1.26 s, 3 H; 1.37 s, 3 H and 1.68 bs, 3 H (6 × CH_3); 2.06 s, 3 H (OAc); 2.12 d, 1 H and 2.84 d, 1 H (2 × H-1, J = 13.9); 3.82 d, 1 H and 4.27 bd, 1 H (2 × H-28, J = 10.3); 4.60 m, 1 H and 4.69 m, 1 H (2 × H-29). Mass spectrum, *m*/*z* (%): 512 (M^+, 1), 484 (1.5), 452 (5.5), 439 (4), 424 (3.5), 409 (2), 342 (2), 43 (100). For $C_{32}H_{48}O_5$ (512.7) calculated: 74.96% C, 9.44% H; found: 74.71% C, 9.21% H.

Partial Esterification of Diacid *VIIa*

A mixture of diacid *VIIa* (200 mg, 0.41 mmol), methanol (20 ml) and concentrated sulfuric acid (1 ml) was refluxed for 1.5 h. The usual work-up and chromatography on silica gel (15 g) in light petroleum–ether (1 : 1) and then in ether afforded diester *VIIIa* (55 mg, 26%), monoester *IXa* (95 mg, 46%) and the starting diacid *VIIa* (40 mg, 20%); the obtained compounds were identical with the authentic compounds obtained as described above.

Oxidative Cleavage of Diosphenol *Vb*

To a solution of diosphenol *Vb* (200 mg, 0.44 mmol) and potassium hydroxide (650 mg) in methanol (30 ml) was added 30% aqueous solution of hydrogen peroxide (1.1 ml); a further portion of the peroxide (1.1 ml) was added after boiling for 20 min. The mixture was then boiled for 1 h, poured into dilute hydrochloric acid and worked up as usual. Chromatography on silica gel (30 g, elution with light petroleum–ether 5 : 3 to 1 : 1 and then with ether) afforded successively monomethyl esters *Xb* (45 mg, 20%) and *IXb* (25 mg, 11%) and diacid *VIIb* (105 mg, 49%).

Diacid VIIb: m.p. 166 – 169 °C (ether–heptane). IR spectrum: 3 625, 3 500 – 2 400, 1 706. Mass spectrum, *m*/*z* (%): 490 (M^+, 1), 472 (3), 459 (9), 441 (6), 430 (9), 413 (9), 385 (77), 373 (14), 343 (9), 155 (42), 55 (100).

Dimethyl ester VIIIb was prepared by treatment of diacid *VIIb* or monomethyl esters *IXb* and *Xb* with ethereal solution of diazomethane; m.p. 149 – 152 °C (ether–methanol), $[\alpha]_D$ −11°. IR spectrum: 3 624, 1 729, 1 719, 1 434, 1 153. ^{1}H NMR spectrum: 0.76 d, 3 H (J = 6.5); 0.84 d, 3 H (J = 6.5); 0.92 s, 3 H; 0.99 s, 3 H; 1.03 s, 3 H and 1.24 s, 6 H (7 × CH_3); 2.35 m, 3 H; 3.31 d, 1 H and 3.77 d, 1 H (2 × H-28, J = 11); 3.61 s, 3 H and 3.65 s, 3 H (2 × OCH_3). Mass spectrum, *m*/*z* (%): 518 (M^+, 1), 500 (1), 487 (9), 445 (5), 417 (6), 399 (15), 387 (7), 343 (32), 169 (60), 102 (100), 95 (98). For $C_{32}H_{54}O_5$ (518.8) calculated: 74.09% C, 10.49% H; found: 73.88% C, 10.21% H.

Monomethyl ester IXb: m.p. 190 – 194 °C (ether–heptane), $[\alpha]_D$ −10°. IR spectrum: 3 624, 3 505 (broad), 1 728, 1 698, 1 435, 1 156. ^{1}H NMR spectrum: 0.76 d, 3 H (J = 6.5); 0.84 d, 3 H (J = 6.5);

0.94 s, 3 H; 0.99 s, 3 H, 1.04 s, 3 H and 1.26 s, 6 H, (7 × CH_3); 2.41 m, 3 H; 3.31 d, 1 H and 3.77 d, 1 H (2 × H-28, *J* = 11); 3.63 s, 3 H (OCH_3). Mass spectrum, *m*/*z* (%): 504 (M^+, 0.1), 486 (0.2), 473 (1), 455 (1), 431 (1.2), 430 (1.5), 417 (5), 399 (20), 343 (20), 169 (45), 88 (19), 95 (100), 43 (99).

Monomethyl ester Xb: m.p. 243 – 247 °C (ether–heptane). IR spectrum: 3 614, 3 515 (broad), 1 740, 1 718, 1 433, 1 145. ^{1}H NMR spectrum: 0.75 d, 3 H (*J* = 6.5); 0.84 d, 3 H (*J* = 6.5); 0.93 s, 3 H; 0.96 s, 3 H; 1.04 s, 3 H; 1.24 s, 3 H and 1.25 s, 3 H (7 × CH_3); 2.38 m, 3 H; 3.31 d, 1 H and 3.78 d, 1 H (2 × H-28, *J* = 11); 3.67 s, 3 H (OCH_3). Mass spectrum, *m*/*z* (%): 504 (M^+, 1), 486 (4), 473 (7), 445 (11), 444 (12), 427 (4), 413 (6), 403 (18), 385 (65), 343 (25), 191 (80), 155 (40), 102 (100).

Acetyl derivative XIb was obtained either from dimethyl ester *VIIIb* by treatment with acetic anhydride in pyridine under conditions described for compound *XIa* or by reaction of a mixture of monomethyl esters *XII* and *XIII* with ethereal diazomethane; m.p. 128 – 132 °C (methanol), $[\alpha]_D$ –17°. IR spectrum: 1 720, 1 434, 1 245, 1 153. ^{1}H NMR spectrum: 0.75 d, 3 H (*J* = 6.3); 0.83 d, 3 H (*J* = 6.3); 0.92 s, 3 H; 0.98 s, 3 H; 1.03 s, 3 H and 1.39 s, 6 H (7 × CH_3); 2.04 s, 3 H (OAc); 2.23 m, 3 H; 3.60 s, 3 H and 3.65 s, 3 H (2 × OCH_3); 3.81 d, 1 H and 4.24 bd, 1 H (2 × H-28, *J* = 10.7). Mass spectrum, *m*/*z* (%): 501 (M^+ – 59, 1), 500 (0.3), 487 (2), 459 (1), 427 (5), 399 (12), 385 (12), 325 (14), 191 (50), 169 (90), 102 (60), 43 (100). For $C_{34}H_{56}O_6$ (560.8) calculated: 72.82% C, 10.06% H; found: 72.57% C, 9.82% H.

28-Acetoxy-2α-hydroxylupan-3-one (*XV*)

A solution of sulfuric acid in methanol (25 ml of 0.05% solution) was added to a solution of ketone *IIIb* (500 mg, 1.03 mmol) and 70% *m*-chloroperoxybenzoic acid (500 mg, 2.03 mmol) in dichloromethane (12.5 ml) and the mixture was set aside at room temperature for 5 h. After addition of ether (50 ml), the solution was successively washed with water, solutions of potassium iodide, sodium thiosulfate and sodium hydrogen carbonate, and with water. After drying over sodium sulfate and evaporation of the solvent, the crude product (460 mg) was dissolved in a mixture of benzene–ether (10 : 1) and the solution was filtered through a small column of silica gel. Crystallization from ether afforded hydroxy ketone *XV* (440 mg, 85%), m.p. 168 – 172 °C, $[\alpha]_D$ –15°. IR spectrum: 3 487, 1 727, 1 703, 1 245. ^{1}H NMR spectrum: 0.76 d, 3 H (*J* = 6.5); 0.84 d, 3 H (*J* = 6.5); 0.93 s, 3 H; 1.10 s, 6 H; 1.15 s, 3 H and 1.18 s, 3 H (7 × CH_3); 2.06 s, 3 H (OAc); 2.47 dd, 1 H (H-1β, *J*(1α, 1β) = 13.0, *J*(1β, 2β) = 6.8); 3.28 bs, 1 H (OH); 3.80 d, 1 H and 4.27 d, 1 H (2 × H-28, *J* = 11); 4.54 dd, 1 H (H-2β, *J*(1α,2β) = 12.7, *J*(1β, 2β) = 6.8). Mass spectrum, *m*/*z* (%): 500 (M^+, 16), 482 (2), 440 (7), 427 (13), 397 (4), 191 (44), 43 (100). For $C_{32}H_{52}O_4$ (500.8) calculated: 76.75% C, 10.47% H; found: 76.47% C, 10.51% H.

Oxidative Cleavage of Hydroxy Ketone *XV*

Methanol (2 ml) and lead tetraacetate (300 mg, 1.15 mmol) were added in two equivalent portions to a solution of hydroxy ketone *XV* (200 mg, 0.4 mmol) in acetic acid (20 ml), the second portion being added after stirring at room temperature for 2 h. After standing for further 15 h at room temperature, the reaction mixture was worked up in the usual manner and the products were separated by chromatography on silica gel (24 g; elution with light petroleum–ether 10 : 3, 5 : 3, and then with ether); yield 50 mg (24%) of anhydride *XIVb* and 120 mg (55%) of a mixture of methyl esters *XII* and *XIII* in the ratio of 2 : 1.

Anhydride XIVb: m.p. 202 – 204 °C (ether–heptane), $[\alpha]_D$ +18.5°. IR spectrum: 1 796, 1 752, 1725, 1 245. ^{1}H NMR spectrum: 0.77 d, 3 H (*J* = 6.5); 0.85 d, 3 H (*J* = 6.5); 0.97 s, 3 H; 1.06 s, 3 H; 1.08 s, 3 H; 1.27 s, 3 H and 1.38 s, 3 H (7 × CH_3); 2.06 s, 3 H (OAc); 2.23 d, 1 H and 2.85 d, 1 H (2 × H-1, *J* = 14); 3.80 d, 1 H and 4.26 d, 1 H (2 × H-28, *J* = 11). Mass spectrum, *m*/*z* (%): 514

(M^+, 2), 454 (15), 441 (13), 426 (4), 411 (4), 397 (3), 191 (56), 43 (100). For $C_{32}H_{50}O_5$ (514.8) calculated: 74.67% C, 9.79% H; found: 74.49% C, 9.53% H. Anhydride *XIVb* was also obtained from diacid *VIIb* by treatment with acetic anhydride in pyridine under conditions described for anhydride *XIVa*.

Mixture of monomethyl esters XII and XIII: IR spectrum: 3 516 (broad), 1 728, 1 700 sh, 1 435, 1 246, 1 155. ^{1}H NMR spectrum: 0.76 d (J = 6.3); 0.84 d (J = 6.3); 0.95 s, 0.99 s and 1.05 s (5 × CH_3); 1.24 s and 1.25 s (2 × CH_3 in isomer *XII*); 1.27 s (2 × CH_3 in isomer *XIII*); 2.05 s, (OAc); 2.3 – 2.5 m; 3.64 s (OCH_3 of isomer *XII*); 3.66 s (OCH_3 of isomer *XIII*); 3.81 d and 4.25 d (2 × H-28, J = 11). Mass spectrum, *m*/*z* (%): 486 (M^+ – 60, 0.5), 473 (0.5), 459 (0.5), 445 (1), 427 (1), 413 (2), 399 (6), 385 (8), 325 (6), 191 (40), 169 (50), 43 (100).

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REFERENCES

1. Baas W. J.: Phytochemistry *24*, 1875 (1985).
2. Baas W. J., van Berkel J. E. M.: Phytochemistry *30*, 1625 (1991).
3. Muthukuda P. M.: Chem. Sri Lanka *2*, 13 (1985); Chem. Abstr. *106*, 116493 (1987).
4. Zaletova N. I., Kluev N. A., Yartseva I. V., Tolkachev O. N.: Khim. Prirod. Soedin. *1987*, 671.
5. Lontsi D., Sondengam B. L., Ayafor J. F.: J. Nat. Prod. Lloydia *52*, 52 (1989).
6. Lontsi D., Sondengam B. L., Martin T. M., Bodo B.: Phytochemistry *30*, 1621 (1991).
7. ElGamal M. H. A., ElTawil B. A. H., Fayez M. B. E.: J. Pharm. Sci. *62*, 1557 (1973).
8. Pradhan B. P., Dutta S. R.: Indian J. Chem., B *23*, 565 (1984).
9. Dutta S. R., Pradhan B. P.: Indian J. Chem., B *22*, 680 (1983).
10. Ganguly G. M.: Tetrahedron *22*, 3597 (1966).
11. Dutta S. R., Pradhan B. P.: Indian J. Chem., B *21*, 575 (1982).
12. Uvarova N. I., Oshitok G. I., Elyakov G. B.: Carbohydr. Res. *27*, 79 (1973).
13. Tietze L. F., Heinzen H., Moyna P., Rischer M., Neunaber H.: Liebigs Ann. Chem. *1991*, 1245.
14. Sejbal J., Klinot J., Budesinsky M., Protiva J.: Collect. Czech. Chem. Commun. *56*, 2936 (1991).
15. Ruzicka L., Rey E.: Helv. Chim. Acta *24*, 529 (1941).
16. Tori M., Matsuda R., Sono M., Kohama Y., Akasawa Y.: Bull. Chem. Soc. Jpn. *61*, 2103 (1988).
17. Cole B., Bentley M. D., Hua Y.: Holzforschung *45*, 265 (1991).
18. ElGamal M. H. A., ElTawil B. A. H., Fayez M. B. E.: Tetrahedron *30*, 4083 (1974).
19. Hanna R., Ourisson G.: Bull. Soc. Chim. Fr. *1967*, 3742.
20. Lugemwa F. N., Huang F.-Y., Bentley M. D., Mendel M. J., Alford A. R.: J. Agric. Food Chem. *38*, 493 (1990).
21. Sejbal J., Klinot J., Vystrcil A.: Collect. Czech. Chem. Commun. *52*, 1052 (1987).
22. Klinot J., Sejbal J., Vystrcil A.: Collect. Czech. Chem. Commun. *54*, 400 (1989).

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