CONVERSION OF BETULIN INTO CAREYAGENOLIDE (2α,3β-DIHYDROXY-18α,19β*H*-URSAN-28,20β-OLIDE)*

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Careyagenolide (*I*) and its respective 2β,3α- and 2β,3β-isomers *XVII* and *XIV* were prepared from 3-oxo-18α,19β*H*-ursan-28,20β-olide (*IX*), accessible from betulin (*II*). The key step of the synthesis was hydroxylation of ketones *IX* and *X* with 3-chloroperoxybenzoic acid and reduction of ketones *XI*—*XIII*.

Recently, Das and Mahato isolated[1] triterpenoid lactone careyagenolide from the leaves of *Careya arborea*. On the basis of spectral data and chemical correlation with Ψ-taraxasterol, the compound has been shown to be 2α,3β-dihydroxy-18α, 19β*H*-ursan-28,20β-olide (*I*). Having in hand suitable starting material and methods for preparation of triterpenoid 2,3-diols (see refs[2-5] and references therein), we prepared careyagenolide and two its isomers, isomeric at C(2) and C(3), from the known[6] 3-oxo-18α,19βH-ursan-28,20β-olide (*IX*). Since the lactone *IX* can be prepared from betulin (*II*) by a sequence of known reactions, this synthesis represents the conversion of betulin (*II*) into careyagenolide (*I*).

The starting compounds were prepared by the already published procedures: the acid-catalysed rearrangement of betulin (*II*) into allobetulin (*III*; 19β,28-epoxy--18α-oleanan-3β-ol) and opening of the ether ring to give heterobetulin (*IV*; 18α, 19β*H*-urs-20-ene-3β,28-diol) were performed as described[7,8]. The acetate *V* with the six-membered ether ring, which had been prepared[6] by a three-step procedure from heterobetulin (*IV*), was obtained by us in one reaction step by heating heterobetulin with *p*-toluenesulfonic acid in acetic acid in a 72% yield. Oxidation of the ether bridge to the lactone one and transformation of the obtained acetate *VII* into the hydroxy derivative *VI* and ketone *IX* was performed according to ref.[6].

One of the approaches to the isomeric 2,3-diols was opening of the 2α,3α- and

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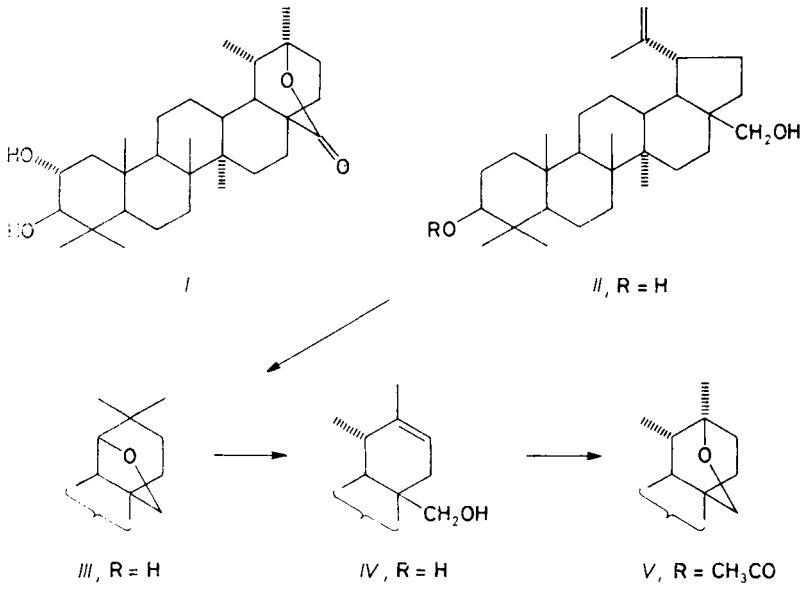
2β,3β-epoxides taking advantage of the known[3] stereochemistry of the reaction. We tried to obtain 18α,19β*H*-urs-2-en-28,20β-olide, the starting compound for the preparation of the epoxides, by way of analogy[9], i.e. by pyrolytic elimination of



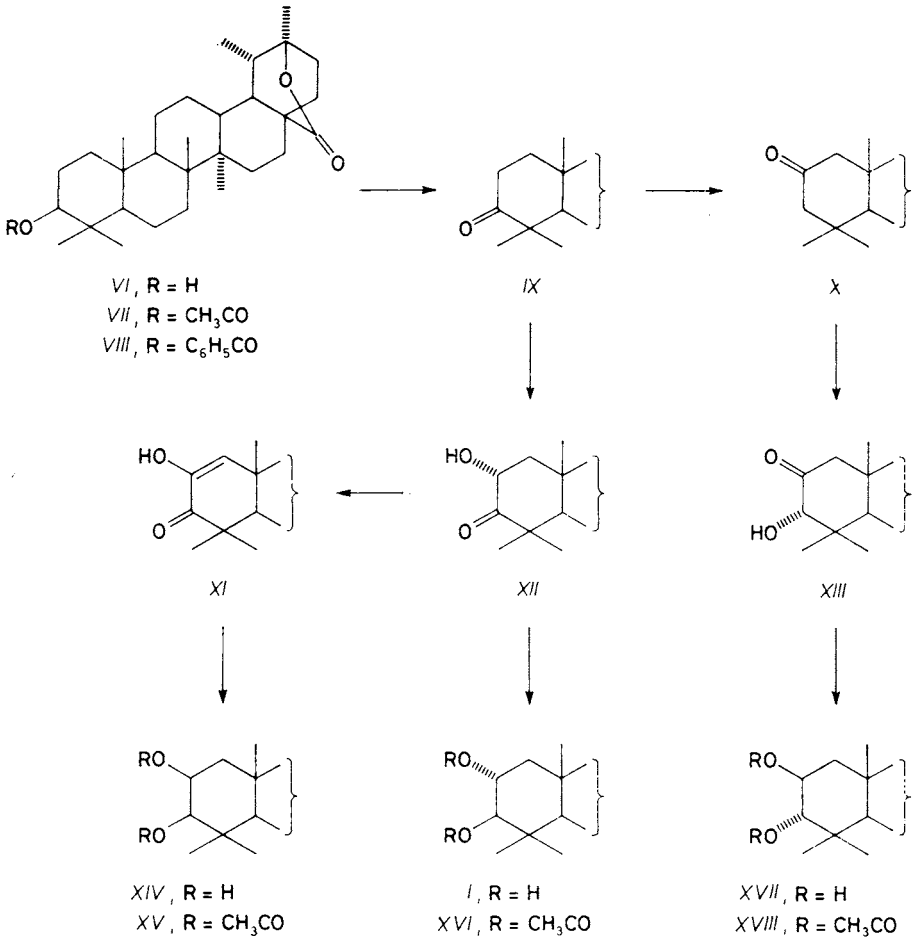
I *II*, R = H

III, R = H *IV*, R = H *V*, R = CH_3CO

benzoic acid from benzoate *VIII*, prepared by reaction of hydroxy derivative *VI* with benzoyl chloride in pyridine. However, on heating to 330–360°C the benzoate *VIII* underwent a more profound decomposition giving a mixture, from which no desired 2(3)-unsaturated derivative was isolated. On the other hand, at lower temperatures no elimination took place.

Therefore, we made use of the recently described[4] direct α-hydroxylation of triterpenoid ketones with peroxy acids in the presence of aliphatic alcohols and small amount of a mineral acid. This method was applied to ketones *IX* and *X*. The 2-ketone *X* was prepared from the 3-ketone *IX* by heating with sulfur in morpholine under conditions[10] of the Willgerodt–Kindler reaction. Reaction of ketone *IX* with 3-chloroperoxybenzoic acid in a mixture of methanol and dichloromethane, containing 0·01% (w/v) of sulfuric acid, afforded 2α-hydroxy-3-ketone *XII*. Similarly, the 2-ketone *X* was hydroxylated to give 3α-hydroxy-2-ketone *XIII* (for a detailed discussion of hydroxylation conditions see ref.[4]). The structure of the hydroxy ketones follows from an analogy[4] and has been confirmed by ^{1}H NMR spectra: the spectrum of *XII* exhibits signals of H-2β (δ 4·54 ddd), OH (δ 3·60 d) and H-1β (δ 2·50 dd); the H-1α signal (δ ~ 1·08 t) was identified by spin decoupling. The coupling constants $J(1\alpha, 2)$ = 12·5 Hz, $J(1\beta, 2)$ = 6·6 Hz, $J(1\alpha, 1\beta)$ = 12·5 Hz and $J(2, OH)$ = 3·4 Hz are compatible with the 2α-hydroxy-3-oxo grouping. The spectrum of isomer *XIII*

displays a singlet of H-3β (δ 4·33) and two doublets of protons at C(1) (δ 2·13 and 2·47; $J(1\alpha, 1\beta) = 17{\cdot}6$ Hz). These data agree well with those for analogous derivatives of 19β,28-epoxy-18α-oleanane[4,5].



Reduction of the 2α-hydroxy-3-ketone *XII* with sodium borohydride afforded 2α,3β-diol *I* as the principal product, reduction of 3α-hydroxy-2-ketone *XIII* led to 2β,3α-diol *XVII*. The hydroxy ketone *XII* was oxidized with air oxygen in an alkaline medium[5] to afford 2,3-dioxo derivative which in solution exists as diosphenol *XI*. The structure *XI* follows from the infrared spectrum, containing bands due to a diketone enol form (3 449, 1 667 and 1 643 cm^{-1}), and from the ^1H NMR spectrum, exhibiting an olefinic proton singlet (δ 6·45) and a hydroxyl proton singlet (δ 5·91). Reduction of diketone *XI* with sodium borohydride gave 2β,3β-diol *XIV*

as the sole product. The diols *I*, *XIV* and *XVII* were converted into the respective diacetates *XVI*, *XV* and *XVIII*.

Configuration of hydroxyl groups in the diols follows from analogies (see refs[2,4,5] and references therein) and from ^{1}H NMR spectra of their diacetyl derivatives. The configuration of isomeric 2,3-disubstituted triterpenoids can be determined from the coupling constant $J(2, 3)$ and $\sum J(1, 2)$ (usually no reliable values of the individual constants $J(1\alpha, 2)$ and $J(1\beta, 2)$ can be obtained from the spectra). The constants $J(2, 3) = 10{\cdot}3$ Hz, $J(1\alpha, 2) = 11{\cdot}6$ Hz and $J(1\beta, 2) = 4{\cdot}7$ Hz, found for the 2α,3β-diacetate *XVI*, $J(2, 3) = 4{\cdot}0$ Hz and $\sum J(1, 2) = 7{\cdot}4$ Hz for 2β,3β-diacetate *XV*, and $J(2, 3) = 8{\cdot}2$ Hz and $\sum J(1, 2) = 12{\cdot}9$ Hz for the 2β,3α-diacetate *XVIII*, are typical of these configurations[5,11]. The mentioned values, as well as chemical shifts of the H-2 and H-3 signals, are in excellent agreement with the data published for analogous diacetates derived from 19β,28-epoxy-18α-oleanane[5,11] and lupane[2]. Compounds *XI*–*XIII*, *XV*, *XVI* and *XVIII* have a singlet of 20α-methyl protons at $\delta \sim 1{\cdot}32$ and a doublet of 19α-methyl protons at $\delta \sim 0{\cdot}99$ ($J = 7$ Hz): these values correspond to the 18α,19β*H*-ursan-28,20β-olide skeleton[12].

The physical data published[1] for careyagenolide and its diacetate are in good accord with our values found for the diol *I* and the diacetate *XVI* prepared in this communication. The observed melting point difference for the diol *I* (see Experimental) is probably caused by decomposition at the melting point which, as we found, depends on the heating rate. For the diacetate *XVI* the melting point is the same as reported[1]. The mass spectra of *I* and *XVI* contain all the published[1] significant ions and in the ^{1}H NMR spectrum of diacetate *XVI* the chemical shifts of the acetate and skeletal methyl groups differ from the published ones less than by 0·01 ppm, the only exception being one of the skeletal methyl signals for which the difference is greater (the singlet at δ 0·930 corresponds either to the singlet at δ 0·97 or δ 0·98 in ref.[1]). Also the chemical shifts and coupling constants of the A-ring protons show a good agreement: however, the doublet of doublets at $\delta \sim 2{\cdot}10$, ascribed[1] to H-18, belongs obviously to H-1β. This signal appears only in the spectrum of diacetate *XVI* whereas it has not been found in the spectra of other 18α,19β*H*-ursan-28,20β-olide derivatives (*XI*–*XIII*, *XV*, *XVIII*).

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Specific rotations were measured in chloroform (*c* 0·3–0·9) on an automatic polarimeter ETL-NPL (Bendix–Ericsson), accuracy ±2°. IR spectra were recorded in chloroform on a PE 684 (Perkin–Elmer) spectrometer; wavenumbers are given in cm^{-1}. Proton NMR spectra were obtained with an FT Varian XL 200 (200 MHz) instrument in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are in ppm (δ-scale), coupling constants (J) in Hz. All parameters were obtained by first order analysis. Mass spectra were measured on a ZAB-EQ (Vacuum Generators) spectrometer, ionizing electrons energy 70 eV, ion source temperature 240°C, inlet

at 215°C. The reaction course was monitored, and purity of the compounds was checked by thin-layer chromatography on silica gel G (Merck; detection by sprayirg with 10% sulfuric acid and heating), or on Silufol foils (Kavalier, Votice; detection by spraying with 10% solution of phosphomolybdic acid in ethanol and heating). Preparative thin-layer chromatography was carried out on silica gel G (Merck); detection with UV-light after spraying with 0·1% methanolic solution of morin. Column chromatography was performed on Silpearl (Kavalier, Votice). Analytical samples were dried at 100°C over phosphorus pentoxide in vacuo.

20β,28-Epoxy-18α,19β*H*-ursan-3β-ol Acetate (*V*)

A solution of heterobetulin[8] (*IV*; 12·4 g) and *p*-toluenesulfonic acid (3 g) in acetic acid (600 ml) was refluxed for 5 h. After cooling, the reaction mixture was poured into water, the precipitate filtered, dissolved in chloroform and the solution dried over sodium sulfate. Two crystallizations from chloroform and methanol afforded 9·75 g (72%) of the title compound *V*, identical with a previously prepared sample[6]; m.p. 281—283°C; $[\alpha]_D$ +52°; reported[6] m.p. 281—283°C, $[\alpha]_D$ +47°.

3β-Benzoyloxy-18α,19β*H*-ursan-28,20β-olide (*VIII*)

A solution of hydroxy derivative *VI* (ref.[6], 0·53 g) and benzoyl chloride (5 ml) in pyridine (30 ml) was set aside at room temperature for 3 days, poured into water and the product was taken up in ether. The ethereal layer was washed with dilute hydrochloric acid, sodium hydrogen carbonate solution and water and filtered through a short column of aluminium oxide. Crystallization from chloroform–methanol gave benzoate *VIII* (0·4 g), m.p. 314—315°C, $[\alpha]_D$ +53°. IR spectrum: 1 729, 1 708, 1 279. For $C_{37}H_{52}O_4$ (560·8) calculated: 79·24% C, 9·35% H; found: 79·26% C, 8·96% H.

2-Oxo-18α,19β*H*-ursan-28,20β-olide (*X*)

A mixture of 3-oxo-18α,19β*H*-ursan-28,20β-olide[6] (*IX*; 0·4 g), morpholine (15 ml) and sulfur (0·1 g) was refluxed for 40 h, sulfur (0·1 g) being added every 4 h. The hot mixture was poured into five-fold amount of 5% sodium sulfide solution. The precipitate was collected, washed with water, dried under diminished pressure, dissolved in chloroform and the solution was filtered through a short column of silica gel (2 g). Chromatography on silica gel (7 g) in light petroleum–benzene (1 : 1) afforded successively: the starting *IX* (30 mg), a mixture of 3-oxo and 2-oxo derivatives *IX* and *X* (90 mg), and 2-oxo derivative *X* (210 mg), m.p. 291—292°C (chloroform–methanol), $[\alpha]_D$ +51°. IR spectrum: 1 730, 1 697. For $C_{30}H_{46}O_3$ (454·7) calculated: 79·25% C, 10·20% H; found: 79·15% C, 10·22% H.

2,3-Dioxo-18α,19β*H*-ursan-28,20β-olide (*XI*)

Air was introduced into a solution of 2α-hydroxy-3-ketone *XII* (160 mg) and potassium hydroxide (1·0 g) in a mixture of benzene (10 ml) and ethanol (20 ml) at room temperature for 6 h. Part of the solvents was distilled off under diminished pressure and the mixture was poured into water, neutralized with dilute hydrochloric acid and extracted with ether. The ethereal solution was washed with water and dried by passing through a layer of alumina. Evaporation of ether and crystallization from chloroform–methanol afforded the diketone *XI* (95 mg), m.p. 261 to 268°C; $[\alpha]_D$ +41°. IR spectrum: 3 583, 3 449, 1 729, 1 667, 1 643. ^{1}H NMR spectrum: 0·909 s, 3 H; 1·001 s, 3 H; 1·005 d, 3 H ($J = 7$); 1·108 s, 3 H; 1·135 s, 3 H; 1·205 s, 3 H and 1·326 s, 3 H (7 × CH_3); 5·91 s, 1 H (OH, isotopic exchange on addition of CD_3OD); 6·45 s, 1 H (H-1). For $C_{30}H_{44}O_4$ (468·7) calculated: 76·88% C, 9·46% H; found: 76·61% C, 9·28% H.

2α-Hydroxy-3-oxo-18α,19β*H*-ursan-28,20β-olide (*XII*)

Compound *IX* (300 mg) and 3-chloroperoxybenzoic acid (300 mg) were dissolved in 0·01% (w/v) solution of sulfuric acid in a 2 : 1 mixture of methanol and dichloromethane (20 ml). After standing at room temperature for 20 h, the reaction mixture was partitioned between ether and water, the ethereal layer was washed with 5% aqueous sodium carbonate and water, dried over sodium sulfate and stripped of the solvents under diminished pressure. Crystallization from chloroform–methanol afforded 2α-hydroxy-3-ketone *XII* (230 mg), m.p. 256–265°C (decomp.), $[\alpha]_D$ +25°. IR spectrum: 3 481, 1 730, 1 701. ^{1}H NMR spectrum: 0·887 s, 3 H; 0·986 s, 3 H; 0·992 d 3 H (J = 7·0); 1·096 s, 3 H; 1·144 s, 3 H; 1·178 d, 3 H (J = 0·7) and 1·318 s, 3 H (7 × × CH_3); ~1·08 t, 1 H (H-1α, J = 12·5 and 12·5); 2·50 dd, 1 H (H-1β, J = 12·5 and 6·6); 3·60 d, 1 H (OH, J = 3·4); 4·54 ddd, 1 H (H-2, J = 12·5, 6·6 and 3·4). For $C_{30}H_{46}O_4$ (470·7) calculated: 76·55% C, 9·85% H; found: 76·20% C, 9·69% H.

3α-Hydroxy-2-oxo-18α,19β*H*-ursan-28,20β-olide (*XIII*)

Compound *X* (160 mg) was converted into 3α-hydroxy-2-ketone *XIII* (140 mg), m.p. 251–260°C (decomp.), (chloroform–methanol), $[\alpha]_D$ +85°, as described for the hydroxy ketone *XII*. IR spectrum: 3 481, 1 729, 1 708. ^{1}H NMR spectrum: 0·818 s, 3 H; 0·899 s, 3 H; 0·965 s, 3 H; 0·995 d, 3 H (J = 7·1); 1·119 s, 3 H; 1·183 s, 3 H and 1·319 s, 3 H (7 × CH_3); 2·13 d, 1 H (H-1α, J = 17·6); 2·47 d, 1 H (H-1β, J = 17·6); 4·33 s, 1 H (H-3). For $C_{30}H_{46}O_4$ (470·7) calculated: 76·55% C, 9·85% H; found: 76·62% C, 9·99% H.

2α,3β-Dihydroxy-18α,19β*H*-ursan-28,20β-olide (*I*)

A suspension of sodium borohydride (100 mg) in methanol (10 ml) was added to a solution of 2α-hydroxy-3-ketone *XII* (160 mg) in benzene (6 ml). After standing at room temperature for 6 h with intermittent shaking, the reaction mixture was diluted with ether and washed successively with water, dilute hydrochloric acid, solution of sodium hydrogen carbonate and water. After drying over sodium sulfate, the solvents were distilled off under reduced pressure and the residue was crystallized from chloroform–methanol, affording diol *I* (125 mg), m.p. 305–310°C or 316–320°C (more rapid heating) with decomposition; $[\alpha]_D$ +18° (reported[1] m.p. 299°C with decomposition). IR spectrum: 3 586, 1 732. Mass spectrum, m/z (%): 472 (M^+, 1), 454 (7), 436 (3), 426 (1), 421 (2), 411 (4), 393 (1·5), 355 (2·5), 261 (5), 235 (8), 219 (10), 205 (17), 189 (20), 187 (18), 55 (100). For $C_{30}H_{48}O_4$ (472·7) calculated: 76·23% C, 10·24% H; found: 76·01% C, 10·20% H.

Diacetate *XVI* was prepared from diol *I* by treatment with a mixture of acetic anhydride and pyridine (1 : 2) at room temperature for 20 h. The reaction mixture was decomposed with ice and extracted with ether, the ethereal solution was washed with dilute hydrochloric acid, sodium hydrogen carbonate solution and water, and dried over sodium sulfate. Diacetate *XVI* melted at 306–308°C (chloroform–methanol), $[\alpha]_D$ +2°. (Reported[1] m.p. 306–308°C.) IR spectrum: 1 735, 1 259, 1 240, 1 369. Mass spectrum, m/z (%): 556 (M^+, 0·5), 496 (0·5), 481 (2), 454 (5·5), 436 (5·5), 421 (2), 411 (1), 393 (2), 261 (18), 247 (1·2), 234 (2·5), 219 (1·5), 203 (6), 187 (15), 119 (15), 55 (22), 43 (100). ^{1}H NMR spectrum: 0·877 s, 3 H; 0·888 s, 3 H; 0·902 s, 3 H; 0·930 s, 3 H; 0·973 d, 3 H (J = 0·3); 0·983 d, 3 H (J = 7·0) and 1·313 s, 3 H (7 × CH_3); 1·98 s, 3 H and 2·05 s, 3 H (2 × CH_3COO); 2·10 dd, 1 H (H-1β, J ~ 12 and ~4·5); 4·73 d, 1 H (H-3, J = 10·3); 5·10 ddd, 1 H (H-2, J = 11·6, 10·3 and 4·7). For $C_{34}H_{52}O_6$ (556·8) calculated: 73·35% C, 9·41% H; found: 73·15% C, 9·28% H.

2β,3β-Dihydroxy-18α,19β*H*-ursan-28,20β-olide (*XIV*)

Using the procedure described for *I*, diketone *XI* (90 mg) was converted into 2β,3β-diol *XIV* (50 mg), m.p. 270–278°C (decomp.) (chloroform–methanol), $[\alpha]_D$ +48°. IR spectrum: 3 623, 3 564, 1 733. For $C_{30}H_{48}O_4$ (472·2) calculated: 76·23% C, 10·24% H; found: 76·35% C, 10·31% H.

Diacetate *XV* was prepared from diol *XIV* as described for the preparation of diacetate *XVI*; m.p. 258–259°C (chloroform–methanol), $[\alpha]_D$ +47°. IR spectrum: 1 734, 1 377, 1 368, 1 259. ^{1}H NMR spectrum: 0·888 s, 3 H; 0·902 s, 3 H; 0·954 s, 3 H; 0·990 d, 3 H (J = 7·1); 1·027 s, 3 H; 1·107 s, 3 H and 1·315 s, 3 H (7 × CH_3); 2·03 s, 3 H and 2·04 s 3 H (2 × CH_3COO); 4·61 d, 1 H (H-3, J = 4·0); 5·33 m, 1 H (H-2, J = 4·0, ~3·7 and ~3·7). For $C_{34}H_{52}O_6$ (556·8) calculated: 73·35% C, 9·41% H; found: 73·48% C, 9·50% H.

2β,3α-Dihydroxy-18α,19β*H*-ursan-28,20β-olide (*XVII*)

Using the procedure described for *I*, 3α-hydroxy-2-ketone *XIII* (100 mg) was converted into 2β,3α-diol *XVII*. Purification by TLC on silica gel in benzene–ether (1 : 1) followed by crystallization from chloroform–methanol, afforded 65 mg of *XVII*, m.p. 263–268°C (decomp.), $[\alpha]_D$ +51°. IR spectrum: 3 582, 1 730. For $C_{30}H_{48}O_4$ (472·7) calculated: 76·23% C, 10·24% H; found: 76·15% C, 10·18% H.

Diacetate *XVIII* was prepared as described for *XVI*; m.p. 315–317°C (chloroform–methanol), $[\alpha]_D$ +54°. IR spectrum: 1 732, 1 256. ^{1}H NMR spectrum: 0·909 s, 3 H; 0·924 s, 6 H; 0·967 s, 3 H; 0·984 d, 3 H (J ~ 7); 1·062 s, 3 H and 1·314 s, 3 H (7 × CH_3); 2·008 s, 3 H and 2·064 s, 3 H (2 × CH_3COO); 4·96 dt, 1 H (H-2, J = 8·2, ~6·5 and ~6·5); 5·09 d, 1 H (H-3, J = 8·2). For $C_{34}H_{52}O_6$ (556·8) calculated: 73·35% C, 9·41% H; found: 73·21% C, 9·40% H.

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