# TRITERPENES. XXIII.*

# DEHYDROGENATION OF DERIVATIVES OF 20(29)-LUPENE WITH MERCURIC ACETATE. III.** DEHYDROBETULINIC ACID

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When 20(29)-lupene derivatives are dehydrogenated with mercuric acetate, dehydro derivatives with lactone (*XXI*) or epoxide (*XXIII*) cycle linked with position 13β were obtained as by-products in low yields. Using ORD and PMR it was shown that in bromoketone *X* and *XI* the conformation of the side chain is stabilised to an appreciable extent not only between positions $C_{(19)}-C_{(20)}$, but also $C_{(20)}-C_{(29)}$. Norketones *VII* and *XIII*, or *VIII* and *XIV* undergo a stereospecific rearrangement under extension of ring E (*XVI–XVIII*) in the presence of bases or acetic anhydride. Double dehydration of α-ketol *XIV* gave the olefinic-acetylenic derivative *XXV* in which the steric interaction of the side chain does not prevent the conjugation of both multiple bonds.

In the preceding communication[1] we investigated the modification of the side chain in dehydrobetulin and made conclusions, based on our results, concerning the structure of this compound. In this paper we describe the preparation of analogous derivatives from dehydrobetulinic acid (*VII*). Their properties corroborate independently the localisation of the lactone ring and contribute to the solution of the side chain conformation.

Using a procedure described earlier[2,3] we obtained from 3-O-acetylbetulinic acid (*I*) 28 → 19β-lactone *V* as the main product in 80–85% yield; in addition to this we also isolated a small amount (0·19%) of isomeric γ-lactone acetate having an isopropenyl side chain. However, in comparison with 28 → 19β-lactone *V* the signals of the side chain in the PMR spectrum of this isomer are distinctly shifted (for $CH_3-\underset{|}{C}=$ : +24·5 Hz, for $CH_2=C\!\!<$ : −125 and −147·5 Hz); thus, this side chain must be under the direct influence of the lactone ring. As this must have the β-configuration preserved and as it is linked with a fully substituted carbon, this isomer might be formulated as 3β-acetoxy-19ξ*H*-20(29)-lupen-28 → 13β-olide (*XXI*), *i.e.*

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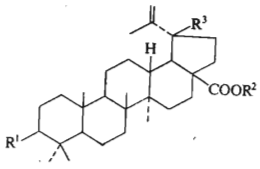
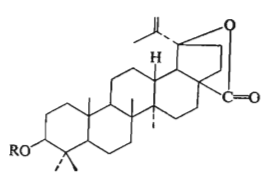
as Allison and his coworkers[2] originally formulated the structure of the main product of dehydrogenation of acid *I* with mercuric acetate. In view of the low yield we were unable to determine whether this isomeric lactone *XXI* can be converted to 28 → 19β-lactone *V*, which, in a positive case, could mean that it is the primary product of dehydrogenation. As we were unable to find conditions under which 28→19β-lactone *V* could be isomerised to 28→13β-lactone *XXI*, it seems that this by-product is rather the result of a side reaction than consecutive one. An analogous isomer could also be expected after dehydrogenation of 3-O-acetylbetulin; however, as we already said in our previous communication[3], we were unable to demonstrate the formation of the isomer with the 13β,28-epoxidic bond after dehydrogenation. Only after subsequent ozonolysis of the main reaction product we isolated[1] in addition to 3β-acetoxy-19β,28-epoxy-30-nor-20-lupanone (*VI*) a small amount (0·68%) of trinorketone of the composition $C_{29}H_{44}O_4$. From its spectral data the following functional groups are evident: 3β-acetoxyl (IR: 1722, 1258, 1030 $cm^{-1}$, PMR: 2·03 p.p.m. and a multiplet of the 3α-proton 4·49 p.p.m.) a keto group vicinal to C—H bonds (IR: 1711, 1418 $cm^{-1}$; PMR: 2·36 p.p.m.), and finally the 28-methylene group on the tetrahydrofuran ring in which its oxygen member is bound to a fully substituted carbon (PMR: 3·52 d + 4·07 dd p.p.m., $J_{gem} = 7$, $J_{1,r.} = 3$ Hz). As we have found[4] that on ozonolysis of 20(29)-lupene derivatives, derivatives of even 20,29,30-trinor-18β-lupan-19-one are formed by side reactions, the structure of 3β-acetoxy-13β,28-epoxy-20,29,30-trinor-19-lupanone (*XXIII*) may be supposed for the above mentioned by-product on the basis of proved functional groups. While trinorketones of the first type, having *cis*-fused rings D and E, display a positive Cotton effect[5,6] and in comparison with the original lupane derivate possess the 14α-methyl group signal shifted upfield (+16 Hz, ref.[4]), the Cotton effect of ketone *XXIII* is opposite ($a = -55$, 328/273 nm), similarly to the shift of the signal of its 14α-methyl group (−9 Hz). From this it follows that cycles D/E are *trans*-fused[7] which is in agreement with the proposed localisation of its epoxide bridge between the positions 13β and 28. From the two mentioned cases it can be judged that the participation of the position $C_{(13)}$ in the dehydrogenation of 20(29)-lupene derivatives with mercuric acetate cannot be completely excluded.

The main product of dehydrogenation of 3-O-acetylbetulinic acid (*I*), *i.e.* lactone *V*, has a much less stable lactone ring than, for example, the derivatives of 18α-oleanan-28→19β-olide[8,9], because it hydrolysed easily under the conditions of alkaline catalysis; direct esterification of the liberated acid with diazomethane gave hydroxy ester *III* which recyclised readily to the starting lactone *V*. Therefore the hydrolysis of the lactone ring must have taken place without further structural changes. *cis*-Configuration of the tertiary hydroxy group and the methoxycarbonyl group at $C_{(17)}$ in ester *III* is evidenced by the strong intramolecular hydrogen bridge (tetrachloromethane $\nu_{(OH)}$ 3480 $cm^{-1}$) and the downfield shift of the signal of the methyl of the ester bond (3·75 p.p.m.).

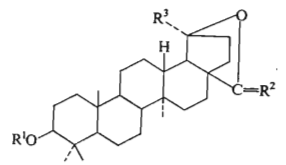
For the subsequent preparation of norketone *VIII* we chose ozonolysis of lactone *V* on the one hand and the oxidation of epoxy derivative *VI* on the other. While the first procedure gave a practically quantitative yield of norketone *VIII*, oxidation of epoxy derivative *VI* gave in addition to ketone *VIII* an appreciable amount of acidic products. After the esterification of this fraction with diazomethane methyl ester of dinor acid *IX* was isolated as the main component. The same dinor derivative is also obtained by oxidation of norketone *VIII* and subsequent esterification of the acid product. Bromination of norketone *VIII* under the conditions found earlier[1,4] also afforded a mixture of bromo ketone *X* and dibromo ketone *XI*. Similarly as in the analogous 19β,28-epoxy derivative in bromo ketone *X* the Cotton effect ($a = -35$, 312/270 nm) is not changed either, as compared with that of the starting ketone *VIII* ($a = -22$, 306/273 nm). In the PMR spectrum its protons on $C_{(29)}$ have different chemical shifts (p.p.m. 4·38 and 4·92) with the geminal coupling constant $J_{gem} = 14{\cdot}3$ Hz. According to[10] from the π-orbital contribution of the carbonyl



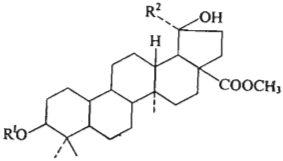

*I*, $R^1 = OAc$, $R^2 = R^3 = H$
*II*, $R^1 = R^3 = OH$, $R^2 = H$
*III*, $R^1 = R^3 = OH$, $R^2 = CH_3$




*IV*, R = H
*V*, R = Ac




*VI*, $R^1 = Ac$, $R^2 = H_2$, $R^2 = COCH_3$
*VII*, $R^1 = H$, $R^2 = O$, $R^3 = COCH_3$
*VIII*, $R^1 = Ac$, $R^2 = O$, $R^3 = COCH_3$
*IX*, $R^1 = Ac$, $R^2 = O$, $R^3 = COOCH_3$
*X*, $R^1 = Ac$, $R^2 = O$, $R^3 = COCH_2Br$
*XI*, $R^1 = Ac$, $R^2 = O$, $R^3 = COCHBr_2$
*XIIa,b*, $R^1 = Ac$, $R^2 = O$, $R^3 = CH(OH)CH_3$

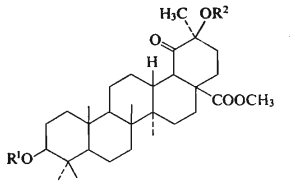



*XIII*, $R^1 = H$, $R^2 = COCH_3$
*XIV*, $R^1 = Ac$, $R^2 = COCH_3$
*XV*, $R^1 = Ac$, $R^2 = CH(OH)CH_3$

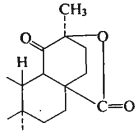
group to $J_{gem}$ the population of the conformer with the eclipsed conformation of the $C_{(20)}$=O and $C_{(29)}$—Br bonds may be deduced; its value, −14·3 Hz, corresponds to 46·8%. Hence, this conformer, in which halogen does not protrude into the active octant, is more stable ($\Delta G^0 = -328$ cal/mol) than both remaining conformers with the eclipsed conformation of $C_{(20)}$=O and $C_{(29)}$—H bonds, in which the halogen atom should more clearly affect the Cotton effect. The analogous result following from these two independent effects justifies the idea of a strong hindrance of the rotation not only around the $C_{(19)}$—$C_{(20)}$ bond, but also around the $C_{(20)}$—$C_{(29)}$ bond in bromoketone *X*. Therefore it is not surprising that the introduction of an additional bromine atom in position $C_{(29)}$ (*XI*) leads to a substantial increase of the amplitude ($a = -147$) and to a bathochromic effect of both extrema (330/281 nm). The reason for this is the relatively fixed conformation of ligands on $C_{(29)}$ in dibromo ketone *XI* where one of the halogen atoms must protrude into the active (negative) octant.

According to an earlier formulation[2] of the structure of dehydrobetulinic acid (*XXI*) norketone *VIII* was also formulated by formula *XXII*; in effort to prove the configuration at $C_{(19)}$ this norketone was submitted to "epimerisation" by sodium tert-butoxide[11]. From the properties of the product formed the conclusion was made that epimerisation really did take place and that the starting norketone *XXII* has the unstable 19α configuration at $C_{(19)}$. As according to the above we proved that the lactone cycle of dehydro derivative *V* is easily opened under the conditions of base catalysed hydrolysis and that it is closed within position $C_{(19)}$, the described reaction of norketone *VIII* cannot have the course mentioned. Therefore we studied more profoundly the course of the base catalysed hydrolysis of keto lactone *VIII*. It was found that under the influence of alkali hydroxide in benzene–ethanol and at laboratory temperature only the 3-O-acetyl group is split off under formation of *VII*; hydrolysis in the same medium (2 hours refluxing) gave a mixture of two hydroxy acids which were esterified with diazomethane and separated chromatographically on alumina. The faster moving ester has two hydroxy groups of which one, secondary (PMR: 3·20 m p.p.m. for 3α-H), is in the position 3β, while the other, tertiary (PMR: 5·415 s p.p.m.; disappears on exchange in deuterium oxide), is alternatively associated with the methoxycarbonyl or C-acetyl group. These facts agree with formula *XIII*. In agreement with this formula acetylation of ester *XIII* with acetic anhydride and pyridine at room temperature gives 3-O-acetyl derivative *XIV* exclusively, the unesterified tertiary hydroxyl of which is a part of a strong intramolecular hydrogen bond (IR, tetrachloromethane: $\nu$(OH) 3456 $cm^{-1}$). In contrast to the starting keto lactones *VII* and *VIII* both keto esters *XIII* and *XIV* produce a positive Cotton effect of medium amplitude, very close to the corresponding 19β-H derivatives[4,6,12]. For the more strongly adsorbed methyl ester the formula *XVI* is considered justified for the following reasons: it also contains a secondary and a tertiary associated hydroxy group, but not the C-acetyl group; instead of the signals of this group the PMR spectrum contains a singlet of the axial methyl group in the grouping $CH_3$—

—C—O (1·36 p.p.m.), see for example[13,14]. This ester also gives on mild acetylation only 3-O-acetyl derivative *XVII* with intramolecularly associated hydroxy group. In dihydroxy derivative *XVI* and in its monoacetate *XVII* a keto group can be further demonstrated which displays a distinctly negative Cotton effect ($a = -106$ (*XVI*), or $-146$ (*XVII*)). As dehydration of monoacetate *XVII* with phosphorus oxychloride in pyridine gives α,β-unsaturated ketone *XX*, the tertiary hydroxy group in the monoacetate *XVII* must be in the α-position with respect to the keto group; in view of the intramolecular hydrogen bond it must also be in equatorial conformation. Further

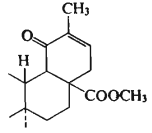



*XVI*, $R^1 = R^2 = H$
*XVII*, $R^1 = Ac$, $R^2 = H$
*XVIII*, $R^1 = R^2 = Ac$



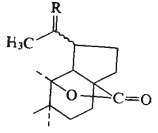

3β-OCOCH$_3$

*XIX*



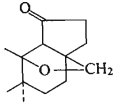

3β-OCOCH$_3$

*XX*



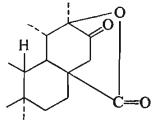

3β-OCOCH$_3$

*XXI*, R = H$_2$
*XXII*, R = O



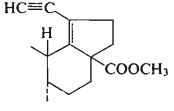

3β-OCOCH$_3$

*XXIII*



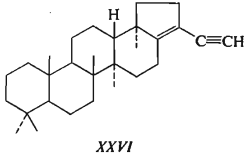

3β-OCOCH$_3$

*XXIV*




3β-OCOCH$_3$

*XXV*




*XXVI*

data corroborating the validity of formula *XVI* follow from the reaction of this dihydroxy ester with acetic anhydride at an elevated temperature; two products were formed of which one is a diacetate with differently bound acetoxy groups, with a methoxycarbonyl group, and the original link of one methyl group in the $CH_3$—|—C—O grouping (1·575 p.p.m.). As the course of the ORD curve of monoacetate |*XVII* and the new diacetate are practically identical, which is also true of the signals of the remaining angular methyl groups, this diacetate must be derived from monoacetate *XVII* by simple acetylation of its tertiary hydroxy group, as is evident from formula *XVIII*. The second product of the reaction of diol *XVI* with acetic anhydride has only a single acetoxy group in the position 3β, further a methyl group in $CH_3$—C—O (PMR: 1·405 p.p.m.), and eventually a δ-lactone cycle connected with a fully substituted carbon atom. Its keto group is evident from a negative Cotton effect, which is, however, rather complex, its course being an inversion of the positive Cotton effect which we found earlier[15] in 3β-acetoxy-21-oxo-18α,19β-*H*-ursan-28→20β-olide (*XXIV*). Therefore we consider the formula *XIX* for this δ-lactone as substantiated. Some of its constants (m.p. 315–317°C, $[\alpha]_D$ −24°, IR: 1752 $cm^{-1}$) are strikingly close to constants given[11] for the supposedly epimerised ketone *VIII* (m.p. 300–303°C, $[\alpha]_D$ −24°, IR: 1750 $cm^{-1}$), formulated as *XXII*. Therefore, it is probable that the quoted authors[11] also obtained lactone *XIX* by the described procedure.

The rearrangement of the skeleton of 19β-hydroxy-30-nor-20-lupanone (*XIII*) to that of 20β-hydroxy-30-nor-18α-olean-19-one (*XVI*) is evidently an analogy of the known (*cf.*[16]) D-homoisomerisation of 17α-hydroxy-20-pregnanone derivatives. In this case the migrating bond is the $C_{(19)}$—$C_{(21)}$ bond. According to mechanistic ideas this is explicable by the fact that the migration of the $C_{(18)}$—$C_{(19)}$ bond would create a transition state with large non-bonding interactions. As the prolongation of the reaction time of the conversion of lactone *VII* (*VIII*) or α-ketol *XIV* (*XIII*) causes the increases in yields of the product with the rearranged skeleton (*XVI* or *XVIII*) (without the formation of $C_{(20)}$-epimer in any reaction phase), the course of the rearrangement in the investigated range is independent of the reaction conditions. From this it follows further that in the starting 19β-hydroxy-30-nor-20-lupane skeleton the conformation of the side chain does not change as is normally supposed[17,18] for the rearrangement of 17α-hydroxy-20-pregnanone derivatives. In this conformation the $C_{(19)}$—OH and $C_{(20)}$=O bonds must be *syn*-clinal, so that the ligands on $C_{(20)}$ might assume the proved conformation in the rearrangement product (*XVI* or *XVIII*).

In contrast to ketol *XVII* the unrearranged ketol *XIV* is relatively resistant to dehydration; only when the conditions are forced the splitting off of two water molecules can take place. The formed dianhydro derivative contains two conjugated multiple

bonds of which one must be the terminal acetylenic bond (IR: 2100, 3330 $cm^{-1}$, PMR: 3·04 s p.p.m.). Therefore, we formulate this dianhydro derivative as methyl ester of 3β-acetoxy-30-norlup-18-en-20(29)-yn-28-oic acid (*XXV*). In view of its formation and structure it can be considered as an analogue of the derivative of 30-norhopane *XXVI* described earlier[19]. As evident from the UV absorption the conjugation of the 18,19 double bond with the ethynyl side chain is not restricted. The loss of conjugation of this double bond with the isopropenyl side chain[20,21] must be caused by a higher degree of hybridisation ($sp^2$) and the substitution of these derivatives at $C_{(20)}$.

In order to complete the series of 30-nor-20ξ-lupanol derivatives we also reduced keto lactone *VIII* with sodium borohydride; a mixture of $C_{(20)}$-epimeric hydroxy derivatives *XIIa,b* was formed in which, according to approximate estimation, both epimers occur in equal proportion. In contrast to this the reduction of hydroxy ester *XIV* with the same reagent took place stereospecifically under formation of one (*XV*) of the possible epimers only.

## EXPERIMENTAL

Melting points were measured on a Kofler block and they are not corrected. Optical rotation was measured in chloroform solutions with a ± 1—2° accuracy. Absorption in the IR region was measured in chloroform unless stated otherwise. Spectrometers ÚPT (ČSAV Brno) and UR-10 (Zeiss, Jena) were used. The ultraviolet spectra were recorded on a Unicam SP-700 spectrometer and PMR spectra on a Varian HA-100 apparatus (in deuteriochloroform at 100 MHz, tetramethylsilane as internal standard). ORD measurements were carried out in dioxan with a Jasco-ORD/UV-5 spectropolarimeter. Alumina for chromatography on columns and thin layers was neutral (Reanal), activity II—III (according to Brockmann). Silica gel, used for the same purpose, was neutral (Spolana, Neratovice). Samples for analysis and spectral measurement were dried over phosphorus pentoxide at 100°C/0·1 Torr for 8—12 hours.

3β-Acetoxy-20(29)-lupen-28 → 19β-olide (*V*)

a) *From* 3-O-*acetylbetulinic acid* (I): Dehydrogenation of 6 g of acid *I* was carried out according to[2,3]. The crude product (5·31 g) was submitted to fractional crystallisation from chloroform–methanol. The combined first and second fractions (4·92 g, 82·2%) represented the pure lactone *V* the analysis, constants, and spectroscopic properties of which were already described by us[3]. The residue of mother liquors (400 mg) was chromatographed on 32 g of silica gel, collecting 25 ml fractions (eluent: benzene). Fractions 3—12 contained another part of lactone *V*, fractions 13—15 were a mixture of lactones *V* and *XXI*. From the residue (35 mg) of fractions 16—19 a treble crystallisation from chloroform–heptane gave 20 mg (0·19%) of an analytically pure specimen of lactone *XXI*, m.p. 285—287°C. IR spectrum: 1777, 1175, 1160 (γ-lactone), 1726, 1257, 1028 ($CH_3COO$), 3100, 1655, 912 ($CH_2$=C) $cm^{-1}$. PMR spectrum: 0·82 (3 × $CH_3$), 0·86, 0·91 (2 × $CH_3$), 1·52 (CH—C=), 2·02 (3β-$CH_3COO$), 4·45 m (3α-H), 6·20m + 6·79 m ($CH_2$=C) p.p.m..

b) *From* 3β,19β-*dihydroxy*-20(29)*lupen*-28-*oic acid* (II): To a solution of 200 mg of crude acid *II* (see below) in 50 ml of benzene 15 ml of acetic acid and 0·05 ml of conc. sulfuric acid were added and the mixture was allowed to stand at room temperature for 14 hours. After dilution with ether and washing with water, saturated sodium hydrogen carbonate solution, and water it was dried over magnesium sulfate, filtered and evaporated to dryness. The residue was dissolved in 5 ml of benzene and chromatographed on 20 g of silica gel. Elution was carried out with benzene–ether 99 : 1, collecting 25 ml fractions. The residue (30 mg) of fractions 7 and 8 was not

further worked up. Fractions 9–14 contained 125 mg of material which after crystallisation from chloroform–methanol gave 95 mg of lactone *V* which is identical with the preparation prepared as under *a*).

Hydrolysis of 3β-acetoxy-20(29)-lupen-28 → 19β-olide (*V*)

To a solution of 170 mg of lactone *V* in 8 ml of benzene 8 ml of a 10% ethanolic potassium hydroxide solution was added and the mixture was refluxed for 1·5 hours. After cooling the mixture was diluted with ether, acidified with dilute hydrochloric acid (1 : 4), and extracted with ether. On subsequent extraction of the ethereal layer with a 5% sodium hydrogen carbonate solution acid material was separated and the remaining ethereal solution containing neutral substances was evaporated to dryness. Crystallisation of the residue (100 mg) from a mixture of chloroform–methanol and from n-heptane gave 65 mg of 3β-*hydroxy*-20(29)-*lupen*-28 → 19β-*olide* (*IV*), m.p. 308–309°C, $[\alpha]_D$ +48·7° (*c* 0·9); lit.[2] m.p. 308–309°C, $[\alpha]_D$ +57°. IR spectrum: 1776, 1185, 1165 (γ-lactone), 1034, 3638 (OH), 1658, 923 ($CH_2$=C) $cm^{-1}$. For $C_{30}H_{46}O_3$ (454·7) calculated: 79·24% C, 10·20% H; found: 79·04% C, 10·12% H. Crude acid *II* (70 mg) isolated from the carbonate solution by acidification was esterified immediately with a diazomethane solution. On crystallisation from a chloroform–methanol mixture (3×) and from n-heptane 35 mg of *methyl ester* of 3β,19β-*dihydroxy*-20(29)-*lupen*-28-*oic acid* (III) were obtained, m.p. 244–246 and 286–291°C, $[\alpha]_D$ +26·4° (*c* 0·50). IR spectrum: 1705, 1440, 1180, 1145 ($COOCH_3$), 1034, 1050, 3460, 3620 (OH), 1648, 913 ($CH_2$=C) $cm^{-1}$. PMR spectrum: 0·755, 0·825, 0·925, 0·965, 0·985 (5 × $CH_3$), 1·775 ($CH_3$—C=), 3·73 ($COOCH_3$), 4·75 m + 5·12 m ($CH_2$=C), 5·21 s (19β-OH, disappearing on deuterium exchange) p.p.m.. For $C_{31}H_{50}O_4$ (486·7) calculated: 76·50% C, 10·36% H; found: 76·38% C, 10·28% H.

3β-Acetoxy-20-oxo-30-norlupan-28 → 19β-olide (*VIII*)

a) *By ozonolysis of lactone* V: Into a solution of 1000 mg of lactone *V* in 100 ml of acetic acid an oxygen current containing 2% of ozone was introduced at room temperature. After 45 minutes no starting lactone *V* could be proved in the mixture; zinc powder (3 g) was added to the mixture which was then stirred at room temperature for 12 hours and further worked up in the conventional manner. The solution of neutral components in benzene was chromatographed on alumina (25 g) collecting fractions of 25 ml each. Benzene–ether 9 : 1 mixture was used for elution. The residue (640 mg) of fractions 3–12 gave on crystallisation from a mixture of chloroform and methanol 560 mg of ketone *VIII*, m.p. 319–321°C, $[\alpha]_D$ −6·2° (*c* 0·45); lit.[2] m.p. 317–319°C, $[\alpha]_D$ +2°, lit.[11] m.p. 301–303°C, $[\alpha]_D$ −9°. IR spectrum: 1721–1715, 1256, 1027 ($CH_3COO$), 1721–1715, 1419 ($CH_3CO$—C), 1784, 1150, 1139 (γ-lactone) $cm^{-1}$. ORD (*c* 0·07): $[\Phi]_{325}$ −1252°, $[\Phi]_{306}$ −2114°, $[\Phi]_{273}$ +132°, $[\Phi]_{250}$ −2628°. PMR spectrum: 0·825 to 0·855 (3 × × $CH_3$), 0·865, 1·05 (2 × $CH_3$), 2·37 ($CH_3CO$—C), 2·02 (3β-$CH_3COO$), 4·46 m (3α-H) p.p.m. For $C_{31}H_{46}O_5$ (498·7) calculated: 74·87% C, 9·04% H; found: 74·76% C, 9·03% H.

b) *By oxidation of* 3β-*acetoxy*-19β,28-*epoxy*-30-*nor*-20-*lupanone* VI: A solution of 400 mg of chromium trioxide in 30 ml of acetic acid was added dropwise over 30 minutes into a solution of 200 mg of ketone *VI* in 30 ml of acetic acid, kept at 45°C and the reaction mixture was allowed to stand at the same temperature for another 2 hours. Then it was left at room temperature for 14 hours. The excess chromium trioxide was reduced with methanol and the mixture was evaporated under reduced pressure to dryness. The residue was extracted with ether and the solution washed repeatedly with water and then fractionated in the usual manner to an acid and a neutral fraction. Crystallisation of the neutral fraction (110 mg) from a chloroform–methanol mixture (5×) gave 70 mg of lactone *VIII*, m.p. 318–320°C, identical with the preparation prepared as under *a*). The residue (90 mg) of the acid fraction was worked up to methyl ester *IX*.

Methyl Ester of 3β-Acetoxy-29,30-dinorlupan-28 → 19β-olide-20-oic Acid (*IX*)

a) *By oxidation of* 3β-*acetoxy*-19β,28-*epoxy*-30-*nor*-20-*lupanone* (VI): The acid fraction after the oxidation of epoxy derivative *VI* (90 mg) was esterified with an ethereal diazomethane solution and then chromatographed on 10 g of silica gel. Elution of fractions (10 ml each) was carried out by a mixture of benzene and ether (98 : 2). From fractions 17—23 a residue (60 mg) was isolated which after two crystallisations from chloroform–heptane gave 50 mg of ester *IX*, m.p. 308 to 310°C, $[\alpha] +18°$ (*c* 0·61). IR spectrum: 1725, 1256, 1032 ($CH_3COO$), 1755, 1447 ($COOCH_3$), 1787, 1152, 1142 (γ-lactone) $cm^{-1}$. PMR spectrum: 0·835 to 0·855 (3 × $CH_3$), 0·875, 0·945 (2 × $CH_3$), 2·03 (3β-$CH_3COO$), 3·80 ($COOCH_3$), 4·47 m (3α-H) p.p.m.. For $C_{31}H_{46}O_6$ (514·7) calculated: 72·34% C, 9·01% H; found: 72·52% C, 8·85% H.

b) *By oxidation of* 3β-*acetoxy*-20-*oxo*-30-*norlupan*-28 → 19β-*olide* (VIII). A solution of chromium trioxide (400 mg) in 30 ml of 85% acetic acid was added dropwise to a stirred solution of 200 mg of ketone *VIII* in 30 ml of acetic acid. The addition, lasting 30 minutes, was carried out at 50°C and the mixture was then kept at the same temperature for 4 hours. The excess chromium trioxide was reduced with methanol and the mixture was evaporated under reduced pressure to dryness. The residue was stirred with 5 ml of dilute hydrochloric acid (1 : 4) and extracted thoroughly with ether. The ethereal layer was washed with water and evaporated to dryness. As according to thin-layer chromatography the residue did not contain neutral components, it was esterified directly with diazomethane and the crude methyl ester was chromatographed as in the preceding procedure *a*). The sample for analysis was vacuum sublimated, m.p. 308—309°C, and it is identical with that prepared under *a*).

Bromination of 3β-Acetoxy-20-oxo-30-norlupan-28 → 19β-olide (*VIII*)

To a solution of 170 mg of ketone *VIII* in 10 ml of chloroform 0·1 ml of a 10% hydrogen bromide solution in acetic acid was added, followed by small portions of a solution of 98·6 mg (1·5 equivalent) of bromine in 10 ml of acetic acid. After 6 hours standing in darkness the mixture was diluted with ether, extracted with water and saturated sodium hydrogen carbonate, dried over magnesium sulfate, and distilled under reduced pressure. The residue was chromatographed on 15 g of silica gel with benzene, collecting 25 ml fractions. The residue of fractions 9—20 (69 mg) was crystallised twice from n-heptane, yielding 57·3 mg of dibromo ketone *XI*, m.p. 276—278°C (decomp.), $[\alpha]_D -1°$ (*c* 0·59). IR spectrum: 1728, 1257, 1027 ($CH_3COO$), 1790, 1150, 1127 (γ-lactone) $cm^{-1}$. ORD (*c* 0·06): $[\Phi]_{400} -1012°$, $[\Phi]_{350} -3864°$, $[\Phi]_{330} -6256°$, $[\Phi]_{325} -5888°$, $[\Phi]_{281} +8464°$, $[\Phi]_{275} +8372°$. PMR spectrum: 0·825 to 0·855 (3 × $CH_3$), 0·87, 1·03 (2 × $CH_3$), 2·02 (3β-$CH_3COO$), 4·46 m (3α-H), 6·485 s (29-H) p.p.m.. For $C_{31}H_{44}Br_2O_5$ (656·5) calculated: 56·71% C, 6·75% H, 24·34% Br; found: 56·22% C, 6·65% H, 24·94% Br. Fractions 21—30 contained according to chromatography on thin layer of silica gel a mixture of bromo ketone *X* and dibromo ketone *XI*. The residue of fractions 31—37 (48·6 mg) was crystallised twice from n-heptane, yielding 29·3 mg of bromo ketone *X*, m.p. 275—277°C, under decomposition, $[\alpha]_D -7$ (*c* 0·23). IR spectrum: 1730—1727, 1257, 1026 ($CH_3COO$), 1730—1727 (C=O), 1788, 1150, 1127 (γ-lactone) $cm^{-1}$. ORD (*c* 0·07): $[\Phi]_{400} -294°$, $[\Phi]_{350} -1325°$, $[\Phi]_{325} -1960°$, $[\Phi]_{315} -2254°$, $[\Phi]_{271} +1176°$, $[\Phi]_{265} +882°$. PMR spectrum: 0·83 to 0·86 (3× $CH_3$), 0·87, 0·945 (2× $CH_3$), 2·02 (3β-$CH_3COO$), 4·20 d +4·375 d, $J_{gem} = 14·5$ Hz (29-$H_2$), 4·48 m (3α-H) p.p.m.. For $C_{31}H_{45}BrO_5$ (577·6) calculated: 64·45% C, 7·85% H; found: 64·44% C, 7·67% H.

Hydrolysis of 3β-Acetoxy-20-oxo-30-norlupan-28 → 19β-olide (*VIII*)

a) *At room temperature*: To a solution of 50 mg of keto lactone *VIII* in 6 ml of benzene potassium hydroxide (80 mg) dissolved in 6 ml of 80% ethanol was added and the mixture was allowed to stand at room temperature for 48 hours. After dilution with ether the mixture was extracted with 5% aqueous tartaric acid solution, then with water. The ethereal layer was dried over magnesium sulfate, filtered and evaporated to dryness. The residue contained according to thin-layer chromatography on silica gel a single component. Therefore, it was directly crystallised from chloroform–n-heptane. Yield 35 mg of hydroxy derivative *VII*, m.p. 263–264°C, $[\alpha]_D$ −12·3° (*c* 0·65). IR spectrum: 1718, 1420 ($CH_3CO$—C), 1785, 1148 (γ-lactone), 1013, 1032, 3610 (OH) $cm^{-1}$. Acetylation of the sample with acetic anhydride and pyridine gave back the starting monoacetate *VIII*.

b) *Under reflux in benzene–ethanol*. A solution of 300 mg of acetate *VIII* in 15 ml of benzene was mixed with 15 ml of a 10% potassium hydroxide solution in 85% ethanol, and the mixture was refluxed for 2 hours. After cooling and acidification with dilute hydrochloric acid (1 : 4) the mixture was extracted with ether and the ethereal layer was washed with water and evaporated to dryness. Esterification with ethereal diazomethane solution gave a mixture of esters which was separated chromatographically on 25 g of silica gel. Elution of fractions (15 ml each) was carried out with benzene–ether (4 : 1). The residue (210 mg) of fractions 4–13 was crystallised from n-heptane, yielding 170 mg of methyl ester *XIII*, m.p. 256–258°C, $[\alpha]_D$ −6° (*c* 0·70). IR spectrum: 1705, 1436, 1194, 1178 ($COOCH_3$), 1705, 1415 ($CH_3CO$—C), 1030, 1040, 3420, 3600 (OH) $cm^{-1}$. ORD (*c* 0·10): $[\Phi]_{400}$ +68°, $[\Phi]_{350}$ +113°, $[\Phi]_{310}$ +1267, $[\Phi]_{306}$ +1312°, $[\Phi]_{265}$ −2896°, $[\Phi]_{255}$ −2800°. PMR spectrum: 0·755, 0·825, 0·91, 0·96, 0·98 (5 × $CH_3$), 2·30 ($CH_3CO$—C), 3·20 m (3α-H), 3·73 ($COOCH_3$), 5·41 bs (19β-OH) p.p.m.. For $C_{30}H_{48}O_5$ (488·7) calculated: 73·73% C, 9·90% H; found: 73·73% C, 9·90% H. Acetylation of 40 mg of hydroxy ester *XIII* was carried out at room temperature with acetic anhydride and pyridine (2 ml, 1 : 1). After conventional working up and crystallisation from n-heptane 30 mg of 3-O-*acetyl derivative* XIV were obtained, m.p. 205–207°C, $[\alpha]_D$ +6·6° (*c* 0·56). IR spectrum: 1718, 1255, 1028 ($CH_3COO$), 1708, 1416 ($CH_3CO$—C), 1708, 1177, 1145 ($COOCH_3$), 3420 (OH) $cm^{-1}$; (tetrachloromethane, *c* 2·9 × $10^{-3}$ mol/l): 3456 $cm^{-1}$. ORD (*c* 0·08): $[\Phi]_{375}$ +60°C, $[\Phi]_{350}$ +121°, $[\Phi]_{305}$ +1145°, $[\Phi]_{270}$ −2290°, $[\Phi]_{263}$ −2412°. PMR spectrum: 0·83 to 0·855 (3 × $CH_3$) 0·915, 0·98 (2 × $CH_3$), 2·02 (3β-$CH_3COO$), 2·30 ($CH_3CO$—C), 3·73 ($COOCH_3$), 4·48 m (3α-H), 5·395 s (19β-OH, disappears after exchange in deuterium oxide) p.p.m.. For $C_{32}H_{50}O_6$ (530·7) calculated: 72·41% C, 9·50% H; found: 72·35% C, 9·41% H. Fractions 14–16 contain according to thin-layer chromatography on silica gel a mixture of esters *XIII* and *XVI*. The residue (20 mg) of fractions 17–20 gave on crystallisation from n-heptane 15 mg of ester *XVI*, m.p. 236–238 and 312–315°C, $[\alpha]_D$ +51° (*c* 0·54). IR spectrum: 1735, 1438, 1179, 1160 ($COOCH_3$), 1713 (C=O), 1028, 1030, 3480 (OH) $cm^{-1}$. ORD (*c* 0·07): $[\Phi]_{400}$ +190, $[\Phi]_{350}$ ±0°, $[\Phi]_{306}$ −2661°, $[\Phi]_{275}$ +7940°, $[\Phi]_{270}$ +7246°. PMR spectrum: 0·765, 0·845 (2 × $CH_3$), 0·945 to 0·975 (3 × $CH_3$), 1·35 ($CH_3$—C—O), 3·65 ($COOCH_3$), 3·21 m (3α-H) p.p.m.. For $C_{30}H_{48}O_5$ (488·7) calculated: 73·73% C, 9·90% H; found: 73·81% C, 9·87% H. Acetylation was carried out as in the case of acetate *XIV*. Crystallisation of the acetate from n-heptane gave 30 mg of 3-O-*acetyl derivative XVII*, m.p. 210–212 and 326–327°C, $[\alpha]_D$ +54·8° (*c* 0·80). IR spectrum: 1725, 1259, 1028 ($CH_3COO$), 1720 – 1715, 1434, 1180, 1148 ($COOCH_3$), 1720 – 1715 (C=O), 3440 (OH) $cm^{-1}$. ORD (*c* 0·065): $[\Phi]_{400}$ +446°, $[\Phi]_{350}$ +223°, $[\Phi]_{325}$ −1441°, $[\Phi]_{307}$ −3670°, $[\Phi]_{275}$ +9660, $[\Phi]_{262}$ +11145°. For $C_{32}H_{50}O_6$ (530·7) calculated: 72·41% C, 9·80% H; found: 72·43% C, 9·58% H. Prolongation of the reaction time of acetate *VIII* with KOH to 3 h of refluxing (other conditions remaining identical) gave derivatives *XIII* and *XIV* in 1 : 3 ratio.

Reactions of Acetates *XIV* and *XVII* with Acetic Anhydride

a) *Reaction of acetate* XVII: A solution of acetate *XVII* (220 mg) in acetic anhydride (10 ml) was refluxed for 7 hours. Acetic anhydride was distilled off under reduced pressure and the residue was dissolved in benzene and chromatographed on 20 g of silica gel. Elution was carried out first with benzene (fractions 1–8, 40 ml each) then benzene–ether 99 : 1 (fractions 9–25), and benzene–ether 95 : 5 (fractions 26–35). The residue of fractions 9–15 (80 mg) was crystallised thrice from chloroform–methanol to give 50 mg of lactone *XIX*, m.p. 315–317°C, $[\alpha]_D$ −24·7° (*c* 0·62). IR spectrum: 1752, 1163, 1081 (δ-lactone), 1727, 1257, 1025 ($CH_3COO$), 1727 (C=O) $cm^{-1}$. ORD (*c* 0·055): $[\Phi]_{400}$ −936°, $[\Phi]_{350}$ −3744°, $[\Phi]_{335}$ −8112°, $[\Phi]_{300}$ +5460°, $[\Phi]_{292}$ +7020°, $[\Phi]_{275}$ +4680°, $[\Phi]_{255}$ ±0°; PMR spectrum: 0·845 (3 × $CH_3$), 0·955 (2 × $CH_3$), 1·405 ($CH_3$—C(=O)—), 2·03 (3β-$CH_3COO$), 4·48 m (3α-H) p.p.m.. For $C_{31}H_{46}O_5$ (498·7) calculated: 74·66% C, 9·30% H; found: 74·56% C, 9·47% H. The residue (130 mg) of fractions 29–33 gave after one crystallisation from methanol and three crystallisations from n-heptane 90 mg of methyl ester *XVIII*, m.p. 258–259°C, $[\alpha]_D$ +62·7° (*c* 0·49). IR spectrum: 1740, 1434, 1179, 1155, 1148 ($COOCH_3$): 1727, 1253, 1026, ($CH_3COO$) $cm^{-1}$. ORD (*c* 0·064): $[\Phi]_{400}$ +364°, $[\Phi]_{333}$ −1040°, $[\Phi]_{317}$ −4056°, $[\Phi]_{280}$ +9048°, $[\Phi]_{270}$ +9880°. PMR spectrum: 0·84 (2×$CH_3$), 0·875, 0·95, 0·975 (3 × $CH_3$), 1·575 ($CH_3$—C(=O)—), 2·045 (20β-$CH_3COO$), 2·035 (3β-$CH_3COO$), 3·71 ($COOCH_3$), 4·47 m (3α-H) p.p.m.. For $C_{34}H_{52}O_7$ (572·8) calculated: 71·29% C, 9·15% H; found: 71·54% C, 8·99% H.

b) *Reaction of acetate* XIV: A solution of 180 mg of acetate *XIV* in 5·5 ml of acetic anhydride was refluxed for 5·5 hours. Further working up was done as under *a*). Yield 40 mg of lactone *XIX* and 100 mg of diacetate *XVIII*, identical with the products prepared as under *a*).

Methyl Ester of 3β-Acetoxy-19-oxo-30-nor-18α-olean-20-en-28-oic Acid (*XX*)

To a solution of 25 mg of acetate *XVII* in 3 ml of pyridine cooled to 0°C a mixture of 3 ml of pyridine and 2 ml of phosphorus oxychloride (also cooled to 0°C) was added and the reaction mixture was allowed to stand at room temperature for 22 hours. It was then heated on a boiling water bath for 15 minutes and cooled. The excess phosphorus oxychloride was decomposed with ice and the precipitated product was extracted with ether. The residue (20 mg), obtained in the usual manner, was dissolved in a mixture of benzene and light petroleum 9 : 1 and adsorbed on 2 g of silica gel. After washing of the column with 60 ml of the same mixture the product was eluted with a mixture of benzene–ether 95 : 5. Crystallisation from chloroform–methanol (once) and n-heptane (twice) gave 7·4 mg of α,β-unsaturated ketone *XX*, m.p. 296–298°C. UV maxima (cyclohexane): $\lambda_{max}$ 226·7 nm, log ε 3·84. IR spectrum: 1726, 1258, 1029 ($CH_3COO$), 1726, 1437, 1197, 1180 ($COOCH_3$), 1686 (C=C—C=O) $cm^{-1}$.

Methyl Ester of 3β-Acetoxy-30-norlup-18-en-20(29)-yn-28-oic Acid (*XXV*)

A solution of 220 mg of α-ketol *XIV* in 20 ml of pyridine was mixed with 10 ml of phosphorus oxychloride under cooling and the mixture was refluxed for 5 hours; after cooling it was poured onto ice, neutralised with 5% sodium carbonate, and extracted with 100 ml of ether. The ethereal extract was washed with dilute hydrochloric acid and water, and then dried over sodium sulfate. After filtering ether was evaporated and the residue (120 mg) dissolved in benzene and chromatographed on 15 g of silica gel. Elution with benzene (10 ml fractions) gave 70 mg of product

(from fractions 3—5) which was crystallised twice from methanol and once from n-heptane. The yield of pure unsaturated ester *XXV* was 55 mg, m.p. 202—204°C, $[\alpha]_D$ +83·2° (*c* 0·60). UV spectrum (cyclohexane): $\lambda_{max}$ 239·8 nm, log ε 4·13, 236 nm, log ε 4·10, and 231 nm, log ε 4·10. IR spectrum: 1735, 1264, 1030 ($CH_3COO$), 1735, 1177 ($COOCH_3$), 3330, 2100 (H—C≡) $cm^{-1}$. PMR spectrum: 0·84 (2 × $CH_3$), 0·89, 0·91, 0·985 (3 × $CH_3$), 2·08 (3β-$CH_3COO$), 3·04 s (H—C≡), 3·685 ($COOCH_3$), 4·49 m (3α-H) p.p.m.. For $C_{32}H_{46}O_4$ (494·7) calculated: 77·69% C, 9·37% H; found: 77·20% C, 9·40% H.

Reduction of 3β-Acetoxy-20-oxo-30-norlupan-28 → 19β-olide (*VIII*) with Sodium Borohydride

To a solution of 130 mg of keto lactone *VIII* in 16 ml of dioxan a solution of sodium borohydride in 12 ml of dioxan and 5 ml of water was added and the mixture allowed to stand at room temperature for 15 hours. The solvents were evaporated in vacuum and the residue was extracted with ether. After further conventional working up the crude product was chromatographed on 31 g of silica gel. Elution was carried out first with benzene (50 ml) and then with benzene–ether 4 : 1, collecting fractions of 20 ml volume each. The residue of fractions 4—7 was crystallised twice from ethyl acetate, then with benzene, yielding 22 mg of hydroxy derivative *XXa*, m.p. above 350°C, $[\alpha]_D$ +37·1° (*c* 1·13). IR spectrum: 1725, 1260, 1032 ($CH_3COO$), 1770, 1187, 1155 (γ-lactone), 3620, 3490 (OH) $cm^{-1}$. PMR spectrum: 0·84 (2× $CH_3$), 0·86, 0·89, 0·94 (3 × $CH_3$), 1·305 d, $J$ = 6·5 Hz ($CH_3$—$C_{(20)}$–H), 2·025 (3β-$CH_3COO$), 4·47 m (3α-H) p.p.m.. For $C_{31}H_{48}O_5$ (500·7) calculated: 74·36% C, 9·66% H; found: 74·28% C, 9·60% H. From the residue (90 mg) of fractions 9—13, which according to thin-layer chromatography on silica gel contained both epimers *XXa,b*, 15 mg of epimer *XXb* were isolated by fractional crystallisation from chloroform–n-heptane; m.p. 282—283°C, $[\alpha]_D$ +41·7° (*c* 0·96). IR spectrum (KBr): 1739, 1250, 1027 ($CH_3COO$), 1769, 1190, 1140 (γ-lactone) $cm^{-1}$. PMR spectrum: 0·84 (2 × $CH_3$), 0·865 (2 × × $CH_3$), 0·94 ($CH_3$), 1·22 d, $J$ = 6·5 Hz ($CH_3$—$C_{(20)}$—H), 2·035 (3β $CH_3COO$), 4·31 bq, $J$ = 6·5 Hz (20-H), 4·48 m (3α-H) p.p.m..

Reduction of Methyl Ester of 3β-Acetoxy-19β-hydroxy-20-oxo-30-norlupan-28-oic Acid (*XIV*) with Sodium Borohydride

To a solution of α-ketol *XIV* (60 mg) in dioxan (10 ml) a solution of sodium borohydride (60 mg) in a mixture of dioxan (6 ml) and water (2 ml) was added. The procedure was then the same as in the preceding reduction. According to the chromatogram of the crude product the reduction gave only one epimer which was isolated by chromatography on 7 g of silica gel. Elution was carried out with benzene–ether 9 : 1, taking 10 ml fractions. The residue (40 mg) of fractions 12—17 was crystallised from chloroform–n-heptane (twice). Yield 25 mg of hydroxy derivative *XV*, m.p. 334—336°C (in sealed capillary). IR spectrum (chloroform): 1727, 1255, 1029 ($CH_3COO$) 1710, 1435, 1196 ($COOCH_3$), 3615, 3515—3450 (OH) $cm^{-1}$; (tetrachloromethane, *c* 3·6 . $10^{-3}$ mol/l): 3628, 3488 (OH) $cm^{-1}$. For $C_{32}H_{52}O_6$ (532·7) calculated: 72·14% C, 9·84% H; found: 72·00% C, 9·80% H.

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