TRITERPENES. XXI.*

3,4-SECO DERIVATIVES OF BETULINIC ACID

J. KLINOT, V. ŠUMANOVÁ and A. VYSTRČIL

Department of Organic Chemistry,
Charles University, Prague 2

Received December 28th, 1970

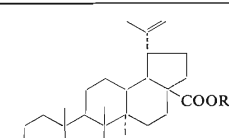
Applying the Beckmann rearrangement of 3-oximino derivatives *IV*, *V*, and *VI* followed by the hydrolysis of the nitriles *VII*, *IX*, and *XI* formed, a series of 3,4-seco derivatives (*VIII*, *XII*–*XVIII*) was prepared, derived from betulinic acid. In acids *VIII*, *XV*, and *XVI* antibacterial activity was detected.

In past years a series of triterpenoid[1–7] and diterpenoid[8,9] 3,4-seco derivatives has been isolated from natural material, which contained at the site of the ring A opening a carboxyl group and a double bond; in some instances the carboxyl group was cyclised to a lactone[10]. Similar types of 3,4-seco derivatives were also found in the group of limonoids[11–13], so it is probable that these seco derivatives play an important role in biological transformations of triterpenes. In connection with the structure of triterpenic antibiotics (fusidic and helvolic acid) it was observed[14,15] that even some steroid and triterpenoid 3,4-seco acids possess antibacterial properties. According to Fried and coworkers[15] the presence of a free carboxyl group in the neighbourhood of another oxygen function or of a double bond is a prerequisite for their antibacterial activity.

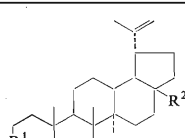
In this paper we describe the preparation of a series of 3,4-seco derivatives of 28-lupanoic acid, which either as such or after the modification of their side chain at $C_{(5)}$ may be considered as potential antibacterial substances. For the preparation of 3,4-seco derivatives literature gives three procedures: *a*) photolytic cleavage of 3-oxo derivatives, leading mainly to saturated derivatives[1,16], *b*) Baeyer–Villiger oxidation of 3-oxo derivatives, giving rise to lactones which can be converted to unsaturated acids by pyrolysis or under the influence of mineral acids[17,18]; *c*) second order Beckmann rearrangement of 3-oximino derivatives, leading to unsaturated nitriles[2,19–21]. Their alkaline hydrolysis gives 3,4-seco-3-acids. In contrast to method *b*) this procedure is convenient even in cases when additional double bonds are present in the molecule, and for this reason it was made use of in our case as well.

As starting material known 3-oxo derivatives[22,23] were employed: betulonic acid methyl ester (*I*), betulonic acid (*II*), and dihydrobetulonic acid methyl ester (*III*). They were prepared from corresponding 3β-hydroxy derivatives on oxidation with chromium trioxide in dimethylformamide[24] and then converted to oximes *IV*–*VI*

* Part XX: This Journal *35*, 3610 (1970).



I, Y = O, R = CH_3
II, Y = O, R = H
III, Y = O, R = CH_3, 20,29-dihydro
IV, Y = NOH, R = CH_3
V, Y = NOH, R = H
VI, Y = NOH, R = CH_3, 20,29-dihydro

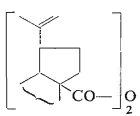


VII, R^1 = CN, R^2 = $COOCH_3$
VIII, R^1 = R^2 = COOH
IX, R^1 = CN, R^2 = COOH
XIII, R^1 = R^2 = $COOCH_3$
XV, R^1 = COOH, R^2 = $COOCH_3$

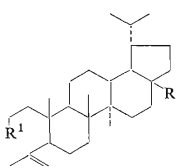
with hydroxylamine hydrochloride in pyridine; oximes *IV* and *VI* were described earlier[22,25]. The oxime of free betulonic acid *V* could not be purified and characterised in the conventional manner because it formed gels occluding large amounts of non-polar solvents and it was used, therefore, for further reactions in its crude state. The Beckmann rearrangement of oximes *IV*–*VI* was carried out by a procedure elaborated by us[19], *i.e.* using toluenesulfonyl chloride in boiling pyridine; such conditions make the yields of nitriles maximum, while lactams are practically not formed[20,21,26]. From oxime *IV* nitrile *VII* was obtained in this manner, which on hydrolysis with 10% sodium hydroxide in boiling ethylene glycol[19] gave the diacid *VIII*; under these conditions the ester group at $C_{(17)}$ also underwent hydrolysis, in addition to the nitrile group. From the oxime of free betulonic acid *V* anhydride *X* was obtained in addition to the nitrile acid *IX*. The structure of this anhydride was proposed on the basis of its infrared spectrum which is similar in character to acyclic anhydrides (strong absorption at 1805, and weaker at 1745 cm^{-1}) and on the basis of alkaline hydrolysis affording diacid *VIII* as in the case of nitrile acid *IX*. By the same procedure 20,29-dihydro derivatives were also prepared: from oxime *VI* nitrile *XI* was prepared which gave diacid *XII* on hydrolysis. Esterification of diacids *VIII* and *XII* with diazomethane gave dimethyl esters *XIII* and *XIV*; in alkaline medium under mild conditions hydrolysis of these dimethyl esters takes place only partially (one ester group only). From the knowledge[27] that the ester groups in the position 17 hydrolyse with great difficulty we deduce for the products thus obtained the structure of 28-methyl esters *XV* and *XVI* with a free carboxyl group in the position 3. Catalytic hydrogenation of dimethyl esters *XIII* and *XIV* gave identical saturated dimethyl ester *XVII*, which demonstrates the relationship between the two series. Similarly, hydrogenation of diacid *XII* led to the saturated diacid *XVIII*.

The mentioned reaction sequence is preparatively advantageous for the preparation of 3,4-seco-3,28-dioic acids with one and also two double bonds, and of their per-

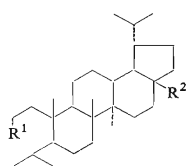
hydro derivatives. When the recently elaborated procedure[28] is applied, the unsaturated compounds *VII*—*XVI* enable preparation of additional derivatives containing oxygen functions at the site of the double bond. According to the above mentioned structural requirements antibacterial activity could be expected only in acids *VIII*, *XV*, and *XVI*. They were tested, therefore, and were found active against *Bacillus subtilis* and *B. cereus*.



X, $R^1 = CN$



XI, $R^1 = CN$, $R^2 = COOCH_3$
XII, $R^1 = R^2 = COOH$
XIV, $R^1 = R^2 = COOCH_3$
XVI, $R^1 = COOH$, $R^2 = COOCH_3$



XVII, $R^1 = R^2 = COOCH_3$
XVIII, $R^1 = R^2 = COOH$

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical rotations were measured in chloroform with ±1—2° precision, the IR spectra were measured in 5—8% chloroform solutions. For chromatography neutral alumina (Reanal, act. II) and silica gel (Spolana, Neratovice, 30—60 μ particle size) were employed. Analytical samples were dried over phosphorus pentoxide at 100°C and 0·1—1 Torr pressure. The starting betulinic acid was obtained by extraction of the planetree bark with methanol according to ref.[29].

Methyl Ester of 3-Oximino-20(29)-lupen-28-oic Acid (*IV*)

To a solution of methyl ester of betulinic acid (3·78 g) in dimethylformamide (300 ml) chromium trioxide (3·80 g) was added in several portions, followed by dropwise addition of sulfuric acid (1 ml). The mixture was allowed to stand at room temperature for 24 hours, then additioned with water, and the product was extracted with chloroform. The extract was washed with water and dried over sodium sulfate. Chloroform was distilled off and the residue crystallised from a chloroform–methanol mixture. Yield: 2·68 g (70%) of derivative *I*, m.p. 163—166°C. A sample of compound *I* was chromatographed on alumina with benzene and crystallised from chloroform–methanol; m.p. 165—166°C; $[\alpha]_D$ + 33° (*c* 1·6). Lit.[22] gives m.p. 165°C; $[\alpha]_D$ +31·4°C.

A solution of derivative *I* (2·35 g) and hydroxylamine hydrochloride (2·50 g) in pyridine (75 ml) was heated at 100°C for one hour and then allowed to stand at room temperature for one day. The solution was diluted with water and the formed precipitate was filtered off, washed with water, and dried at 100°C. Crude oxime *IV* was obtained (2·35 g) which after crystallisation from methanol had m.p. 237—240°C; $[\alpha]_D$ −10° (*c* 1·7). Lit.[22] gives m.p. 238°C. IR spectrum: 3 600, 3 275 (N—OH), 1 650, 894 (C=CH_2), 1 728, 1 440, 1 150, 1 136 cm^{-1} ($COOCH_3$). For $C_{31}H_{49}NO_3$ (483·7) calculated: 76·97% C, 10·21% H, 2·90% N; found: 76·87% C, 10·41% H, 3·17% N.

3-Oximino-20(29)-lupen-28-oic Acid (*V*)

Betulinic acid (5·07 g) was oxidized with chromium trioxide (5·07 g) in dimethylformamide (80 ml) under addition of sulfuric acid (0·6 ml) as in the preceding experiment. The product was dissolved in ethanol and filtered through a layer of charcoal. Ethanol was distilled off and the residue, weighing 4·84 g (95%), had m.p. 235—240°C (Lit.[22] gives for betulonic acid m.p. 249—250°C). Betulonic acid (*II*) thus obtained was converted to its oxime (*V*) on standing in the presence of hydroxylamine hydrochloride (5 g) in pyridine (150 ml) for 76 hours. The crude oxime *V* weighed 4·19 g (86%) and it was used without further purification for the subsequent rearrangement. The attempts at purification of oxime *V* by crystallisation were unsuccessful; the oxime is only slightly soluble in common organic solvents and both in hot solutions and after cooling it forms voluminous gels which retain large quantities of solvents (benzene, ether, methanol). On chromatography of the sample on silica gel by elution with benzene–ether (9 : 1) fractions in the form of gels were formed again, which after evaporation of the solvent under reduced pressure gave a residue melting at 228—229°C.

3-Oximino-28-lupanoic Acid Methyl Ester (*VI*)

Applying the same methods as in the case of the preparation of oxime *IV*, methyl ester of dihydrobetulonic acid *III* (3·22 g, see[23]) was converted to its oxime *VI* (3·10 g; 96%) which after crystallisation from methanol had m.p. 256—260°C (decomp.); lit.[25] gives m.p. 252—253°C. $[\alpha]_D$ −40° (*c* 0·7). IR spectrum: 3 600, 3 270 (NOH), 1 725, 1 438, 1 150, 1 135 cm^{-1} ($COOCH_3$). For $C_{31}H_{51}NO_3$ (485·7) calculated: 76·65% C, 10·58% H, 2·88% N; found: 76·94% C, 10·81% H, 2·71% N.

Rearrangement of Oxime *IV*

A solution of oxime *IV* (2·37 g) and toluenesulfonyl chloride (7 g) in pyridine (50 ml) was refluxed for 5 hours. The reaction mixture was diluted with water, the product extracted with chloroform, and the extract washed with dilute hydrochloric acid (1 : 4), 5% sodium carbonate, and water, and dried over sodium sulfate. Chloroform was distilled off and the residue dissolved in benzene and filtered over aluminum oxide. After the evaporation of benzene crystallisation from a mixture of chloroform and methanol gave 1·26 g (52%) of nitrile *VII*, m.p. 149—150°C; $[\alpha]_D$ +22° (*c* 1·8). IR spectrum: 2 260 (CN), 1 650, 890 (C=CH_2), 1 730, 1 440, 1 162, 1 139 cm^{-1} ($COOCH_3$). For $C_{31}H_{47}NO_2$ (465·7) calculated: 79·95% C, 10·17% H, 3·01% N; found: 79·84% C, 10·29% H, 3·20% N.

Rearrangement of Oxime *V*

A solution of oxime *V* (3·90 g) and toluenesulfonyl chloride (10 g) in pyridine (100 ml) was worked up as above (omitting the extraction with sodium carbonate). The product was filtered through 100 g of silica gel. Elution with benzene (50 ml) gave 0·65 g of anhydride *X*, m.p. 217 to 223°C. Crystallisation from cyclohexane gave a material in the form of needles, m.p. 84—86°C, which solidified after melting to melt again at 227—229°C. At 160—170°C another change of crystal modification was observed; crystallisation from a mixture of cyclohexane–n-hexane gave little plates of m.p. 232—234°C; $[\alpha]_D$ +13 (*c* 2·6). IR spectrum: 2260 (CN), 1650, 896 (C=CH_2), 1805, 1745 cm^{-1} (COOCO). For $C_{60}H_{88}N_2O_3$ (885·3) calculated: 81·39% C, 10·01% H, 3·16% N; found: 81·24% C, 9·86% H, 3·31% N. Further elution with 50 ml of benzene gave 1·55 g of a mixture of substances *IX* and *X*, and next 350 ml of benzene eluted 0·40 g of chromatographically pure nitrile acid *IX*. The analytical sample obtained on crystallisation from

chloroform–methanol had m.p. 228–231°C; $[\alpha]_D$ +26° (*c* 0·6). IR spectrum: 2260 (CN), 2500 to 3000, 1715 (COOH), 1650, 900 cm^{-1} (C=CH_2). For $C_{30}H_{45}NO_2$ (451·7) calculated: 3·10% N; found: 3·20% N.

Rearrangement of Oxime *VI*

Applying the same procedure as for oxime *IV*, nitrile *XI* was obtained from oxime *VI* (2·94 g); after chromatographic purification on alumina and crystallisation of the product from chloroform–methanol 1·91 g (65%) of product were obtained, m.p. 132–134°C; $[\alpha]_D$ – 10° (*c* 0·7). IR spectrum: 2260 (CN), 1645, 900 (C=CH_2), 1730, 1440, 1165, 1140 ($COOCH_3$). For $C_{31}H_{49}NO_2$ (467·7) calculated: 79·60% C, 10·56% H, 3·00% N; found: 79·65% C, 10·54% H, 3·05% N.

3,4-Seco-4(23),20(29)-lupadiene-3,28-dioic Acid (*VIII*)

A mixture of nitrile *VII* (1·20 g), sodium hydroxide (5 g) and ethylene glycol (50 ml) was refluxed for 5 hours. The solution was acidified with hydrochloric acid and the separated precipitate of the crude diacid *VIII* (1·20 g) was filtered off under suction, washed with water, dried, and dissolved in ether. The solution was washed with dilute hydrochloric acid and the acids were extracted with 5% sodium carbonate solution. The extract was acidified with hydrochloric acid and extracted with ether. Ether was distilled off and the residue crystallised from a mixture of ether and n-hexane, to give diacid *VIII* melting at 245–246°C (change of the crystal modification at 180–190°C without visible melting). When the sample was put into the block at 220°C it melted and solidified, to melt again at 243–246°C; $[\alpha]_D$ +25° (*c* 0·7). IR spectrum: 2500 to 3000, 1716 (COOH), 1650, 895 cm^{-1} (C=CH_2). For $C_{30}H_{46}O_4$ (470·7) calculated: 76·55% C, 9·85% H; found: 76·70% C, 10·01% H.

In the same manner nitrile acid *IX* (0·46 g) gave 0·43 g (93%) of diacid *VIII*, and anhydride *X* (0·45 g) gave 0·40 g (89%) of diacid *VIII*. The identity of both these preparations with the sample mentioned above was proved on the basis of their melting points, mixture melting points, optical rotations, and IR spectra.

Dimethyl Ester of 3,4-Seco-4(23),20(29)-lupadiene-3,28-dioic Acid (*XIII*)

The diacid *VIII* (1·91 g) was esterified with diazomethane in an ethereal solution. The product was dissolved in a light petroleum–benzene mixture (10 : 1) and filtered through alumina. Yield 1·70 g (89%) of amorphous, chromatographically pure dimethyl ester, which after crystallisation from ethanol had m.p. 109–111°C; $[\alpha]$ +22° (*c* 0·7). IR spectrum: 1735, 1440, 1162, 1139 ($COOCH_3$), 1650, 895 cm^{-1} (C=CH_2). For $C_{32}H_{50}O_4$ (498·7) calculated: 77·06% C, 10·11% H; found: 77·20% C, 10·30% H.

28-Methyl Ester of 3,4-Seco-4(23),20(29)lupadiene-3,28-dioic Acid (*XV*)

A mixture of dimethyl ester *XIII* (0·91 g), 1M methanolic KOH (4 ml), and benzene (30 ml) was refluxed for 3 hours and then acidified with dilute hydrochloric acid and extracted with ether. The extract was washed with water and dried over sodium sulfate. After filtration ether was evaporated and the residue crystallised from a mixture of ether, n-hexane, and methanol. The obtained methyl ester *XV* (0·70 g, 77%) had m.p. 232–234°C; $[\alpha]_D$ +22° (*c* 1·6). IR spectrum: 2500–3000, 1720 (COOH), 1720, 1440, 1160, 1135 ($COOCH_3$), 1650, 895 cm^{-1} (C=CH_2). For $C_{31}H_{48}O_4$ (484·7) calculated: 76·81% C, 9·98% H; found: 76·98% C, 10·07% H.

3,4-Seco-4(23)-lupene-3,28-dioic Acid (*XII*)

Proceeding as in the preparation of diacid *VIII*, diacid *XII* was obtained (1·79 g, 94%) by hydrolysis of nitrile *XI* (1·91 g). The acid was crystallised from a mixture of ether and n-hexane; m.p. 286—288°C; $[\alpha]_D$ −6° (*c* 1·8). IR spectrum: 2500—3000, 1715 (COOH), 1650, 900 cm^{-1} (C=CH_2). For $C_{30}H_{48}O_4$ (472·7) calculated: 76·22% C, 10·24% H; found: 76·25% C, 10·18% H.

Dimethyl Ester of 3,4-Seco-4(23)-lupene-3,28-dioic Acid (*XIV*)

Esterification of diacid *XII* (0·96 g) with diazomethane and crystallisation of the product from methanol gave dimethyl ester *XIV* (0·79 g, 82%), m.p. 127—129°C, $[\alpha]_D$ −10° (*c* 2·9). IR spectrum: 1735, 1445, 1165, 1136 ($COOCH_3$), 1646, 895 cm^{-1} (C=CH_2). For $C_{32}H_{52}O_4$ (500·7) calculated: 76·75% C, 10·47% H; found: 76·49% C, 10·52% H.

28-Methyl Ester of 3,4-Seco-4(23)-lupene-3,28-dioic Acid (*XVI*)

To a solution of dimethyl ester *XIV* (0·33 g) in 25 ml of benzene 3 ml of 1M methanolic KOH were added and the mixture allowed to stand at room temperature for 7 days. After working up as in the section on the preparation of ester *XV* and crystallisation from an ether–n-hexane–methanol mixture ester *XVI* was obtained (0·22 g, 67%) of m.p. 223—225°C; $[\alpha]_D$ −8° (*c* 1·6). IR spectrum: 2500—3000, 1718 (COOH), 1718, 1440, 1165, 1135 ($COOCH_3$), 1640, 895 cm^{-1} (C=CH_2). For $C_{31}H_{50}O_4$ (486·7) calculated: 76·50% C, 10·36% H; found: 76·71% C, 10·55% H.

Dimethyl Ester of 3,4-Seco-3,28-lupanedioic Acid (*XVII*)

A solution of dimethyl ester *XIII* (0·24 g) in acetic acid (30 ml) was hydrogenated on Adams catalyst for 4 hours. The mixture was diluted with water and the product extracted with ether. The extract was washed with a 5% sodium carbonate solution and with water, and dried over sodium sulfate. After filtration and evaporation of the solvent the residue was dissolved in benzene and the solution was filtered through alumina. Benzene was distilled off and the residue crystallised from light petroleum and then from an ether–methanol mixture. Yield: 0·20 g (83%) of a dimorphous dimethyl ester *XVII*, either in the form of needles of m.p. 101—103/134—136°C, or in the form of prisms of m.p. 135—137°C; $[\alpha]_D$ −18° (*c* 0·7). IR spectrum: 1735, 1440, 1165, 1138 cm^{-1} ($COOCH_3$). For $C_{32}H_{54}O_4$ (502·8) calculated: 76·44% C, 10·83% H; found: 76·59% C, 10·90% H.

Using the same method as above dimethyl ester *XIV* (0·17 g) was hydrogenated to afford 0·16 g (94%) of dimethyl ester *XVII*, m.p. 101—104°/134—135°C (methanol); $[\alpha]_D$ −19° (*c* 0·6). The identity with the sample prepared as above was proved by mixture melting point determination and IR spectra measurement.

3,4-Seco-3,28-lupanedioic Acid (*XVIII*)

A solution of diacid *XII* (0·20 g) in ether (50 ml) was hydrogenated on an Adams catalyst for 2 hours. The mixture was filtered through silica gel and ether was distilled off. The residue was crystallised from an ether–n-hexane mixture. Diacid *XVIII* was obtained in 75% yield (0·15 g), m.p. 293—294°C; $[\alpha]_D$ −20° (*c* 0·7). IR spectrum: 2500—3000, 1710 cm^{-1} (COOH). For $C_{30}H_{50}O_4$ (486·7) calculated: 75·90% C, 10·62% H; found: 75·91% C, 10·64% H.

The infrared spectra were measured by Mrs M. Podzimková and the analyses were carried out by Mrs J. Kohoutová and Mrs B. Šperlichová of the Analytical Department of our Institute (head Dr J. Zelinka). The authors thank Dr M. Blumauerová, Microbiological Institute, Czechoslovak Academy of Sciences, Prague, for antibacterial tests.

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Translated by Ž. Procházka.