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3,4-SECO ACIDS OF 18α-OLEANANE SERIES WITH MODIFIED SIDE CHAIN AT $C_{(5)}$

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A series of 3,4-seco acids (*V, XI, XVI, XXI, XXV*) substituted at $C_{(4)}$ by another oxygen-containing function has been prepared from aldehydes *III* and *XIX* which were obtained on oxidation of the unsaturated nitrile *I*. In the case of acids *V* and *XVI* both $C_{(4)}$-isomers have been obtained and their configuration was derived from infrared spectra of esters *VI* and *XVII*. In the case of derivatives *V* and *VI* the configuration was confirmed by the synthesis of one of the isomers (*VIb*) from ketone *XIII* which was prepared from diacid *XI*.

Some 3,4-seco acids derived from steroids and tetracyclic triterpenes possess antibacterial activity; their effect and also probably the mechanism of activity on microorganisms is similar to that of antibiotics of the fusidic acid type[1-4]. Several active derivatives were also found among pentacyclic triterpenoids[3,5]. A common structural feature of the mentioned derivatives, relevant for their antibacterial activity, is the presence of a free carboxyl and an oxygen containing function or a double bond in its vicinity[3]. This paper describes the preparation of analogous 3,4-seco acids derived from the pentacyclic triterpene 19β,28-epoxy-18α-oleanane, which contain an additional oxygenated function (OH, C=O, COOH) in the side chain attached to $C_{(5)}$. As the starting material the known[6,7] seconitrile *I* was employed; for the modification of the isopropenyl chain the methods described in our previous papers[8,9] were used which are based mainly on acid catalysed rearrangement of the epoxy derivative *II* to a mixture of aldehydes *III*, isomeric at $C_{(4)}$.† The degradation of the unsaturated nitrile *I* to a mixture of hydroxy nitriles *IV* and their hydrolysis to a mixture of hydroxy

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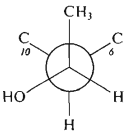
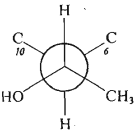
† Derivatives differing in configuration at $C_{(4)}$ are indicated by the same number; if both isomers were obtained in a pure state, they are differentiated by letters *a* and *b*.

acids *V*, have already been described[8]. Chromatography of this mixture on silica gel now gave both isomeric hydroxy acids *Va* and *Vb* which were characterised as methyl esters *VIa* and *VIb*. Methyl ester *VIb* was identical with the ester obtained earlier[8] by crystallisation from a mixture of esters *VI*. On oxidation of hydroxy acid *Va* keto acid *VII* was prepared methyl ester *VIII* of which was also obtained on oxidation of methyl ester *VIb*. Isomeric hydroxy acids *V* differ in their behaviour during melting (240°C): acid *Vb* gives lactone *IX* quantitatively, while acid *Va* does not lactonise under these conditions. On the basis of these facts configuration 4*S* may be proposed for the isomer *Vb*, because it is evident from a model that with this isomer no interaction of the methyl group at $C_{(4)}$ with the axial 10β-methyl group takes place. In the case of the (4*R*)-isomer *Va* this interaction evidently prevents lactonisation. The infrared spectra of methyl esters *VI* (Table I) lead to the same conclusion: isomer *VIb* forms an intramolecular hydrogen bond, while isomer *VIb* does not. A similar type of hydrogen bond between the hydroxy and the ester groups which are separated by a larger number of carbon atoms was observed, for example, in



A



B

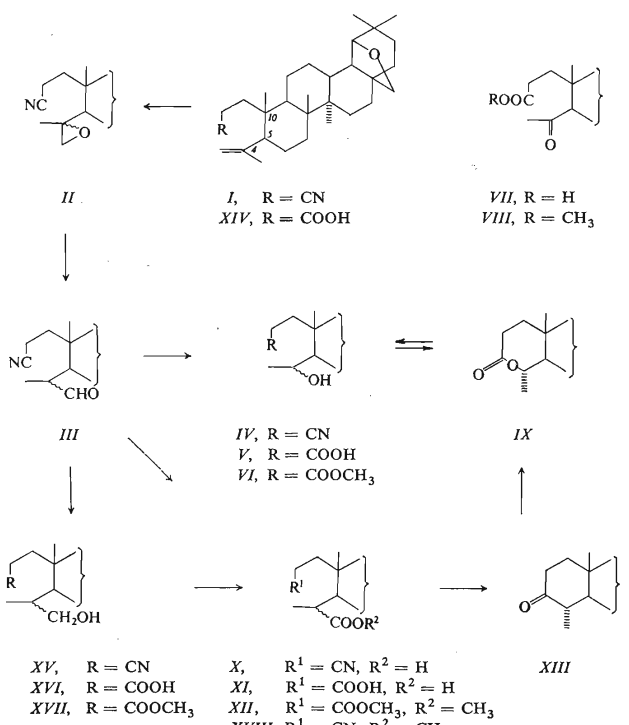
methyl ester of labdanolic acid[10]. From the point of view of the conformation of the $C_{(4)}-C_{(5)}$ bond it may be considered that from the three possible staggered conformers the hydrogen bond will be formed only in the conformer with the hydroxy group (+)-synclinal with respect to $C_{(10)}$. This conformer *A* is in (4*R*)-derivatives

TABLE I
Frequencies of OH-Stretching Vibrations (cm^{-1})

Ester		*VIa*	*VIb*	*XVIIa*	*XVIIb*
ν(OH) cm^{-1}	free	3 621	3 620	3 637	3 636
	bonded	—	3 570, 3 500	3 530	—

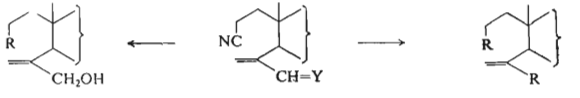
Measured on a grating spectrophotometer Unicam SP 700 in 10^{-2}M solutions in tetrachloromethane. At this concentration intermolecular hydrogen bonding does not yet take place (*cf.*[10]).

VIa made less favourable by the methyl group interaction with the substituents at $C_{(6)}$ and $C_{(10)}$, especially by the strong interaction with the 10β-methyl group. In contrast to this, in the (4*S*)-isomer *VIb* this interaction does not take place and the conformer *B*, suitable for the formation of a hydrogen bond, evidently represents the form with the lowest energy.



The proposed configuration of derivatives *V* and *VI* was further confirmed by synthesis of lactone *IX* and methyl ester *VIb* by the following procedure: epoxy derivatives *II* were isomerized with sulfuric acid in acetic acid and the formed alde-

hydes *III* were oxidized with chromium trioxide to nitrile acids *X* from which a mixture of diacids *XI* was obtained on alkaline hydrolysis. The latter were esterified to methyl esters *XII*. During this procedure $C_{(4)}$-isomers were not separated at any stage and the mixture of acids *XI* was made use of for the next reaction. Heating this mixture with acetic anhydride in the presence of potassium cyanide according to[11] afforded six-membered ketone *XIII*. As this ketone does not undergo isomerisation it must be the thermodynamically more stable isomer with an equatorial 4α-methyl group (see[12,13]). The same ketone was also obtained in low yield from the unsaturated acid[6] *XIV* on epoxidation and subsequent acid catalysed cyclisation; similar cyclisations of epoxy acids to 4α-methyl-3-oxo derivatives have already been described[14,15]. According to the melting point and optical rotation ketone *XIII* is identical with 24-norallobetulone, obtained[16] from natural 23-hydroxybetulin. On Bayer–Villiger oxidation with *m*-chloroperbenzoic acid under conditions when the isomerisation of the methyl group in the position 4 does not take place[17], lactone *IX* was prepared from ketone *XIII*; hydrolysis of lactone *IX* and its esterification gave methyl ester *VIb* (4*S*). Lactone *IX* was recently obtained[18] during the oxidation of allobetulone with peracids.



XX, R = CN
XXI, R = COOH
XXII, R = $COOCH_3$

XIX, Y = O
XXIII, Y = NOH

XXIV, R = CN
XXV, R = COOH
XXVI, R = $COOCH_3$

Another series of seco-derivatives was prepared from the mixture of aldehydes *III*. Its reduction with sodium borohydride gave a mixture of hydroxy nitriles *XV* which was separated chromatographically to isomers *XVa* and *XVb*. On alkaline hydrolysis hydroxy acids *XVIa* and *XVIb* were obtained which were characterised as methyl esters *XVIIa* and *XVIIb*, which again differ by their ability to form intramolecular hydrogen bonds (Table I). Although in this case the situation is more complicated with respect to derivatives *VI*, we suppose that the same conformer as in the case of esters *VI* is involved in the hydrogen bond formation, and therefore we propose the 4*S* configuration for the isomer forming the hydrogen bond (*XVIIa*) while the second isomer (*XVIIb*) should have configuration 4*R*. Hydroxynitriles *XVa* and *XVb* were further transformed by oxidation with chromium trioxide and subsequent esterification to methyl esters *XVIIIa* and *XVIIIb*. These were already obtained earlier[9] after oxidation of the nitrile with peracids. By this procedure derivatives

with the hydroxymethyl group (*XV, XVI, XVII*) and with the carboxyl group (*X, XVIII*) in the position $C_{(4)}$ were correlated, so that the proposed configurations are also valid for carboxyl derivatives.

The preparation of seco derivatives with preserved double bond in the side chain was carried out from α,β-unsaturated aldehyde *XIX* obtained by oxidation of nitrile *I* with selenium dioxide[9].

By reduction of aldehyde *XIX* with sodium borohydride hydroxy nitrile *XX* was formed as the main product which was transformed to hydroxy acid *XXI* and methyl ester *XXII*. From the mother liquors after the isolation of hydroxy nitrile *XX* saturated methyl ester *XVIIb* was also obtained on hydrolysis and esterification; thus, during the reduction with hydride a partial saturation of the double bond also took place. The unsaturated aldehyde *XIX* was further transformed to oxime *XXIII* which was dehydrated with acetic anhydride to dinitrile *XXIV*. Its hydrolysis gave diacid *XXV*, characterised as dimethyl ester *XXVI*, the structure of which was corroborated by PMR spectra.

According to preliminary test the acids *Va, XI, XVIb* and *XXI* are active against *Bacillus subtilis* and *B. cereus, Klöckera apiculata* and *K. africana*. A more detailed discussion of the antibacterial activities of the described 3,4-seco acids will be published elsewhere.

EXPERIMENTAL

The melting points were determined on a Kofler block. Optical rotations were measured in chloroform on an automatic polarimeter ETL-NPL (Bendix-Ericsson) with a ±1—2° accuracy. The infrared spectra were measured in chloroform on an UR-20 spectrophotometer (Zeiss, Jena, GDR) and a model of the Institute of Apparatus Technique, Czechoslovak Academy of Sciences (Brno). The PMR spectra were measured in deuteriochloroform, using tetramethylsilane as internal standard, on a Varian HA-100 apparatus. For chromatography neutral alumina (Reanal, act. II) and silica gel (Spolana, Neratovice) were used. For the washing of solutions aqueous 5% sodium carbonate and dilute hydrochloric acid (1 : 4) were employed. Drying was carried out over anhydrous sodium sulfate. Methyl esters were prepared with ethereal diazomethane solution. Analytical samples were dried over phosphorus pentoxide at 100°C and 0·1—1 Torr for 8—16 hours.

Hydroxy Acids *V*

A mixture of isomeric acids *V* obtained according to[8] was chromatographed on a hundredfold amount of silica gel in benzene. Benzene–ether (9 : 4) mixture eluted hydroxy acid *Va*, m.p. 242—246°C (chloroform–methanol–cyclohexane). For $C_{29}H_{48}O_4$ (460·7) calculated: 75·60% C, 10·50% H; found: 75·61% C, 10·30% H. Methyl ester *VIa* crystallised from a chloroform–n-hexane mixture; m.p. 191—193°C, $[\alpha]_D$ +44° (*c* 1·0). IR spectrum: 1740, 1445, 1175 ($COOCH_3$), 1030 (COC) cm^{-1}. For $C_{30}H_{50}O_4$ (474·7) calculated: 75·90% C, 10·62% H; found: 75·65% C, 10·59% H. Further elution with a mixture of benzene and ether (9 : 5) and crystallisation from a mixture of chloroform, methanol and cyclohexane hydroxy acid *Vb* was obtained, m.p. 239—241°C and after resolidification, 290—293°C. According to thin-layer chromatography on silica gel the sample melting at 240°C corresponds to lactone *IX*. For $C_{29}H_{48}O_4$ (460·7) calculated: 75·60% C, 10·50% H; found: 75·58% C, 10·66% H. Methyl ester *VIb* crystallised from methanol; m.p. 198—200°C, $[\alpha]_D$ +45° (*c* 0·5). Identity with an authentic sample[8] was proved by mixted melting point and IR spectra. IR spectrum: 1732, 1455, 1180 ($COOCH_3$), 1033 (COC) cm^{-1}.

Keto Acid *VII*

A solution of hydroxy acid *Va* (70 mg) and chromium trioxide (40 mg) in acetic acid (6 ml) was allowed to react at room temperature for 20 hours. Methanol and water were then added and the mixture extracted with ether. The extract was washed with a sodium hydrogen carbonate solution and water and dried. After evaporation of ether the residue (55 mg) was crystallised from a mixture of chloroform and n-hexane. Yield 30 mg of keto acid *VII*, m.p. 234—238°C. IR spectrum: 3200—2500, 1765, 1715 (COOH and CO), 1030 (COC) cm^{-1}. For $C_{29}H_{46}O_4$ (458·7) calculated: 75·94% C, 10·11% H; found: 76·08% C, 9·98% H.

Methyl Ester *VIII*

Methyl ester *VIb* (20 mg) was dissolved in warm acetone (10 ml), cooled to room temperature, additioned with 0·1 ml of Jones reagent, and allowed to stand for 5 minutes. The mixture was worked up as above and the residue crystallised from methanol, affording methyl ester *VIII* (12 mg), m.p. 180·5—182°C, $[\alpha]_D$ +37° (*c* 0·9). IR spectrum: 1710 (CO), 1737, 1440, 1177 ($COOCH_3$), 1030 (COC) cm^{-1}. For $C_{30}H_{48}O_4$ (472·7) calculated: 76·22% C, 10·24% H; found: 76·34% C, 10·50% H. The same methyl ester of m.p. 181—182°C (methanol) was obtained on esterification of the keto acid *VII*. Identity of both preparations was confirmed by mixture melting point and IR spectra.

Diacids *XI*

To a solution of epoxy derivative *II* (11·8 g, prepared according to[9] without purification by crystallisation) in acetic acid (250 ml) sulfuric acid was added (20 ml) and the mixture allowed to stand at room temperature for 20 h. Chromium trioxide (2 g) was then added in several portions and the mixture allowed to stand for 1·5 h. The excess reagent was decomposed with methanol. The mixture was diluted with water, extracted with chloroform, the extract was washed with water and sodium carbonate solution, and then dried. Evaporation of chloroform gave 10 g of a non-crystalline mixture of nitrile acid *X*. The same results were obtained if after the reaction of epoxide *II* with sulfuric acid the mixture of aldehydes *III* was first isolated and then oxidized. A mixture of nitrile acids *X* (5·20 g), potassium hydroxide (8 g) and ethanol (250 ml) was refluxed for 19 h. It was then acidified with hydrochloric acid and the separated precipitate was filtered off and the filtrate extracted with ether. The above precipitate was added to the ethereal extract and the solution formed was washed with water. After evaporation of the ether 4·60 g of a mixture of diacids *XI* were obtained. A sample for analysis was prepared by chromatography on silica gel with ether and crystallisation from acetone; m.p. 299—302°C (decomposition), $[\alpha]_D$ +65° (*c* 0·3). For $C_{30}H_{48}O_5$ (488·7) calculated: 73·73% C, 9·90% H; found: 73·80% C, 9·77% H. The mixture of dimethyl esters *XII* had after chromatography (alumina, elution with benzene) and crystallisation from chloroform–methanol m.p. 130—134°C, $[\alpha]_D$ +53° (*c* 0·6). IR spectrum: 1734, 1439, 1170 ($COOCH_3$), 1030 (COC) cm^{-1}. For $C_{32}H_{52}O_5$ (516·7) calculated: 74·37% C, 10·14% H; found: 74·60% C, 10·04% H.

Ketone *XIII*

A) A mixture of diacids *XI* (2·15 g) potassium cyanide (0·4 g) and acetic anhydride (60 ml) was refluxed for 11·5 h. Acetic anhydride was then distilled off under reduced pressure and the residue extracted with ether, the ethereal solution was washed with 10% potassium hydroxide solution and water and dried. Ether was distilled off and the residue (1·8 g) was chromato-

graphed on alumina (60 g) with benzene. With the first 100 ml ketone *XIII* (0·7 g) was eluted, m.p. 218—220°C (dichloromethane–n-hexane), $[\alpha]_D$ +83° (*c* 0·6). Literature[14] gives m.p. 214 to 215°C, $[\alpha]_D$ +84°. IR spectrum 1708 (CO), 1030 (COC) cm^{-1}. For $C_{29}H_{46}O_2$ (426·7) calculated: 81·63% C, 10·87% H; found: 81·35% C, 10·96% H. Later, more polar fractions have not been identified.

B) A solution of acid *XIV* (1·1 g, see[6]) and perbenzoic acid (0·3 g) in chloroform (50 ml) was allowed to stand at 0°C for one day. After washing with sodium hydrogen carbonate solution and water the solution was dried and the solvent evaporated. Yield 1·05 g. A part of the product (0·85 g) was dissolved in chloroform (15 ml) and a mixture of acetic acid (25 ml) and sulfuric acid (2·5 ml) was added to the solution. The mixture was allowed to stand at room temperature for one day, then additioned with water and chloroform, and the chloroform layer was washed with sodium carbonate and water. Chloroform was distilled off and the residue (0·8 g) chromatographed on alumina as under *A*). Yield 0·15 g of ketone *XIII* of m.p. 215—218°C (acetone), $[\alpha]_D$ +80° (*c* 0·7). IR spectrum was identical with that of the preparation described under *A*).

Lactone *IX*

A) A solution of ketone *XIII* (30 mg) and *m*-chloroperbenzoic acid (30 mg) in chloroform (0·6 ml) was allowed to stand at room temperature for 18 h. It was then diluted with chloroform, washed with sodium carbonate solution and water and dried. Crystallisation of the residue from cyclohexane gave lactone *IX* (25 mg) of m.p. 290—293°C (decomposition), $[\alpha]_D$ +46° (*c* 0·7). IR spectrum: 1727 (CO), 1035 (COC) cm^{-1}. The spectrum is similar but not quite identical with that of the lactone published earlier[8]. For $C_{29}H_{46}O_3$ (442·7) calculated: 78·68% C, 10·47% H; found: 78·52% C, 10·58% H. On hydrolysis of lactone *IX* (20 mg) with potassium hydroxide (0·5 g) in a mixture of benzene (2 ml) and methanol (5 ml) under reflux for 6 hours methyl ester *VIb* (15 mg) was obtained after conventional working up and subsequent esterification with diazomethane. M.p. 200—202°C (methanol). Identity with an authentic specimen was confirmed by mixed melting point and IR spectra.

B) Hydroxy acid *Vb* (10 mg) was heated at 250—255°C for 5 min. The crystals formed were dissolved in benzene and chromatographed on alumina (2 g). Elution with ether gave lactone *IX* (8 mg), m.p. 290—292°C (decomposition), the IR spectrum of which was identical with the spectrum of the sample described under *A*).

Hydroxy Nitriles *XV*

A mixture of aldehydes *III* (1·42 g, see[9]), sodium borohydride (0·6 g), chloroform (15 ml) and methanol (10 ml) was allowed to stand at room temperature for 3·5 h. After dilution with water, acidification with hydrochloric acid and extraction with chloroform the extract was washed with a sodium carbonate solution and water and dried. After filtration chloroform was distilled off and the residue dissolved in benzene and chromatographed on alumina (120 g) with benzene–ether mixture (10 : 1). Repeated crystallisation of the eluted product from chloroform–methanol and chloroform–n-hexane gave hydroxy nitrile *XVa* (170 mg); m.p. 226—228°C, $[\alpha]_D$ +30° (*c* 0·7). For $C_{30}H_{49}NO_2$ (455·7) calculated: 79·07% C, 10·84% H; found: 78·94% C, 11·05% H. The same solvent mixture eluted further a mixture of derivatives *XVa* and *XVb*. Elution with benzene–ether mixture (1 : 1) and repeated crystallisation of the eluted product from methanol and a mixture of chloroform and methanol gave hydroxy nitrile *XVb*, (65 mg) of m.p. 229—231°C, $[\alpha]_D$ +37° (*c* 0·7). For $C_{30}H_{49}NO_2$ (455·7) calculated: 79·07% C, 10·84% H; found: 79·23% C, 10·82% H.

Oxidation: A solution of derivative *XVa* (50 mg) and chromium trioxide (25 mg) in acetic acid (15 ml) was allowed to stand at room temperature for 20 h. After conventional work-up and esterification with diazomethane methyl ester *XVIIIa* (35 mg) was obtained which on crystallisation from methanol gave needles of m.p. 160—162°C or prisms of m.p. 172—174°C; $[\alpha]_D$ +64° (*c* 0·7). According to mixture melting point and IR spectrum it is identical with an authentic specimen[9]. By the same procedure methyl ester *XVIIIb* was obtained from hydroxy nitrile *XVb*. The ester melted at 178—180°C (n-hexane) and the m.p. remained undepressed on admixture of an authentic specimen[9].

Hydroxy Acids *XVI*

A mixture of hydroxy nitrile *XVa* (0·15 g), sodium hydroxide (0·7 g) and ethylene glycol (7 ml) was refluxed for 5 h. It was diluted with water, acidified with hydrochloric acid and extracted with ether. The ethereal layer was washed with water, dried, and ether evaporated. The residue was dissolved in benzene and chromatographed on silica gel. On elution with a benzene–ether mixture (9 : 4) and crystallisation from chloroform–methanol–n-hexane mixture hydroxy acid *XVIa* was obtained (40 mg) of m.p. 273—276°C. For $C_{30}H_{50}O_4$ (474·7) $^1/_2$ CH_3OH calculated: 74·65% C, 10·68%H; found: 74·71% C, 10·70% H. Methyl ester *XVIIa* had m.p. 207—209°C (methanol). IR spectrum: 1737, 1445, 1180 ($COOCH_3$), 1032 (COC) cm^{-1}. For $C_{31}H_{52}O_4$ (488·7) calculated: 76·18% C, 10·72% H; found: 76·17% C, 10·47% H. By the same procedure hydroxy nitrile *XVb* (160 mg) was transformed to hydroxy acid *XVIb* (60 mg), which was obtained by chromatography and crystallisation from methanol and chloroform–methanol–n-hexane mixture in a pure state. M.p. 268—270°C. For $C_{30}H_{50}O_4$ (474·7) $^1/_2$ CH_3OH calculated: 74·65% C, 10·68% H; found: 74·90% C, 10·66% H. Methyl ester *XVIIb*, after crystallisation from methanol, melted at 172—173°C, then solidified and remelted at 200—201°C. IR spectrum: 1740, 1445, 1176 ($COOCH_3$), 1030 (COC) cm^{-1}. For $C_{31}H_{52}O_4$ (488·7) calculated: 76·18% C, 10·72% H; found: 76·35% C, 10·56% H.

Hydroxy Nitrile *XX*

To a solution of aldehyde *XIX* (0·22 g, see[9]) in a mixture of benzene (20 ml) and methanol (10 ml) sodium borohydride (0·20 g) was added and the mixture allowed to react at room temperature for 2 h. After working up as in the case of hydroxy nitrile *XV* and crystallisation of the product from a chloroform–methanol and chloroform–ether mixture hydroxy nitrile *XX* was obtained (120 mg) which had m.p. 238—240°C, $[\alpha]_D$ +51° (*c* 0·4). IR spectrum: 3620, 3420 (OH), 2250 (CN), 3100, 1645 (C=CH_2), 1035 (COC) cm^{-1}. For $C_{30}H_{47}NO_2$ (453·7) calculated: 79·42% C, 10·44% H; found: 79·18% C, 10·52% H.

Hydroxy Acid *XXI*

A mixture of hydroxy nitrile *XX* (110 mg), sodium hydroxide (0·5 g) and ethylene glycol (5 ml) was refluxed for 3·5 h and then processed as in the case of the acid *XVI*. The product was esterified with diazomethane, adsorbed from a benzene solution to alumina (7 g) and chromatographed with benzene–ether mixture (19 : 1). Methyl ester *XXII* (35 mg) was obtained which had m.p. 158—159°C (n-hexane), $[\alpha]_D$ +50° (*c* 0·8). IR spectrum: 3618 (OH), 3097, 1647 (C=CH_2), 1736, 1444, 1181 ($COOCH_3$), 1035 (COC) cm^{-1}. For $C_{31}H_{50}O_4$ (486·7) calculated: 76·50% C, 10·36% H; found: 76·60% C, 10·69% H. By the same procedure (*i.e.* hydrolysis, esterification and chromatography) saturated methyl ester *XVIIb* was obtained in addition to methyl ester *XXII* from the mother liquors after crystallisation of hydroxy nitrile *XX*; M. p. 170°C/198—199°C

(n-hexane), undepressed with an authentic sample. On boiling of methyl ester *XXII* with 2% methanolic potassium hydroxide for 2·5 hours hydroxy acid *XXI* of m.p. 258—260°C (ether) was obtained after the conventional work-up. For $C_{30}H_{48}O_4$ (472·7) calculated: 76·22% C, 10·24% H; found: 76·40% C, 10·38% H.

Dinitrile *XXIV*

A solution of aldehyde *XIX* (0·4 g) and hydroxylamine hydrochloride (0·4 g) in pyridine (20 ml) was heated at 100°C for 3 h. Water was added and the mixture extracted with ether. The extract was washed with hydrochloric acid and water and dried. After evaporation of the solvent the residue was dissolved in benzene and chromatographed on alumina (20 g). Elution with ether and crystallisation from a mixture of dichloromethane and n-hexane gave oxime *XXIII* (0·34 g), m.p. 200—201·5°C, $[\alpha]_D$ +89% (*c* 0·7). IR spectrum: 3570, 3370, 1625, 1603, 955 (C=C—C==NOH), 1031 (COC) cm^{-1}. For $C_{30}H_{46}N_2O_2$ (466·7) calculated: 77·20% C, 9·94% H, 6·00% N; found: 77·07% C, 9·93% H, 6·22% N. A mixture of oxime *XXIII* (0·27 g) and acetic anhydride (20 ml) was refluxed for 3 h. Acetic ahydride was decomposed with water and the mixture extracted with ether. The ethereal extract was washed with a sodium carbonate solution and water and dried. Ether was distilled off and the residue dissolved in benzene and the solution filtered through alumina. Yield 160 mg of dinitrile *XXIV*, m.p. 244—246°C (cyclohexane), $[\alpha]_D$ +58° (*c* 1·0). IR spectrum: 2240, 2220 (CN), 1617, 940 (C=CH_2), 1030 (COC) cm^{-1}. For $C_{30}H_{44}N_2O$ (448·7) calculated: 80·30% C, 9·89% H, 6·24% N; found: 80·50% C, 10·15% H, 6·21% N.

Diacid *XXV*

A mixture of dinitrile *XXIV* (220 mg), sodium hydroxide (1·1 g) and ethylene glycol (11 ml) was refluxed for 8 h. It was then diluted with water, acidified with hydrochloric acid and extracted with ether. The ethereal solution was extracted with a sodium carbonate solution and the extract was acidified with hydrochloric acid. The precipitated diacid was extracted with ether and the extract washed with water, dried and evaporated. Crystallisation of the residue from ether gave diacid *XXV* (150 mg), m.p. 239—242°C (on rapid heating it melts at 198—203°C, solidifies and remelts at 237—241°C). IR spectrum: 2400—3400, 1715, 1710 (COOH), 1627 (C=C), 1037 (COC) cm^{-1}. For $C_{30}H_{46}O_5$ (486·7) calculated: 74·03% C, 9·53% H; found: 74·00% C, 9·44% H. Dimethyl ester *XXVI* was chromatographed on alumina (elution with n-hexane–benzene 1 : 1) and crystallised from methanol. M.p. 183—184°C, $[\alpha]_D$ +57° (*c* 0·7). IR spectrum: 1735, 1720, 1445, 1175 ($COOCH_3$), 1630 (C=C), 1036 (COC) cm^{-1}. PMR spectrum (in p.p.m., δ-scale): 0·77 (2 × CH_3), 0·91, 0·93 and 1·005 (3 × CH_3); 3·43 d and 3·77 bd, J = 8 Hz (28-H_2), 3·52 (19-H), 3·62 and 3·70 (2 × $COOCH_3$); 5·48 bs and 6·21 bs (C=CH_2). For $C_{32}H_{50}O_5$ (514·7) calculated: 74·67% C, 9·79% H; found: 74·87% C, 9·71% H.

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