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Lichen Triterpenoids. IV.[1] The Structures of Leucotylic Acid and Methyl Isoleucotylate, an Acid-induced Isomer of Methyl Leucotylate

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The structure of leucotylic acid, isolated together with leucotylin (I) and zeorin (II) from a lichen *Parmelia leucotyliza* Nyl., has been established as 16β,22-dihydroxy-hopan-23-oic acid (III) on the basis of chemical and physicochemical investigation. Furthermore, methyl isoleucotylate, produced in addition to methyl leucotylidienate (VI) under acid treatment of methyl leucotylate (IV), has been elucidated as VII isomeric to IV at the C-21 geometry and hence possessing an isohopane carbon framework. The isomerization procedure from IV to VII has also been briefly discussed.

The structures of two triterpenoid alcohols, leucotylin and zeorin isolated from a lichen *Parmelia leucotyliza* Nyl., have been established as I[1,3,4] and II[1,5,6,7] respectively both possessing the hopane skeleton. Further investigation of the same lichen, especially collected in some parts of the Kinki district of Japan, have led us to isolate a new acidic triterpenoid termed leucotylic acid along with zeorin and leucotylin. As mentioned previously,[3] the yield of this acid in comparison with that of leucotylin varied depending upon the place where the lichen was collected. Thus, the lichen obtained at Nose or Sugio in Osaka-fu was found to contain leucotylic acid in a good yield, while leucotylin in a lesser yield. On the other hand, the lichen from the Izu peninsula was revealed to contain a less amount of leucotylic acid but rich leucotylin. This paper presents the full details on the structure of leucotylic acid(III)[8] and furthermore on the structure of methyl isoleucotylate(VII)[9] obtained by acid treatment of methyl leucotylate(IV).

The Structure of Leucotylic Acid

Leucotylic acid(III), $C_{30}H_{50}O_4$, mp 259—260°, $[\alpha]_D$+330° ($CHCl_3$), exhibited the hydroxyl (3380(sh), 3200 cm^{-1}) and carboxyl (1710(sh), 1690 cm^{-1}) absorption bands in the infrared (IR) spectrum(Nujol), a positive Liebermann-Burchard color test, and the negative property to tetranitromethane. These properties have suggested leucotylic acid to be a saturated triterpenoid.

On methylation with diazomethane III gave methyl leucotylate(IV), $C_{31}H_{52}O_4$, mp 298—301°, which possesses the hydroxyl and carbomethoxyl functions as revealed by its IR spectrum(3200, 1720, and 1240 cm^{-1}). Acetylation of IV with acetic anhydride and pyridine

1) Part III: I. Yosioka, T. Nakanishi, H. Yamauchi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 147 (1972).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) I. Yosioka, T. Nakanishi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 279 (1969).
4) T. Nakanishi, T. Fujiwara, and K. Tomita, *Tetrahedron Letters*, **1968**, 1491.
5) I. Yosioka, T. Nakanishi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 291 (1969).
6) T. Nakanishi, H. Yamauchi, T. Fujiwara, and K. Tomita, *Tetrahedron Letters*, **1971**, 1157.
7) I. Yosioka, T. Nakanishi, znd I. Kitagawa, *Tetrahedron Letters*, **1971**, 1157.
8) I. Yosioka, T. Nakanishi, and E. Tsuda, *Tetrahedron Letters*, **1966**, 607 (Preliminary account on the subject).
9) I. Yosioka, M. Yamaki, T. Nakanishi, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 2227 (Preliminary account on the subject).

yielded smoothly methyl 16-O-acetyl-leucotylate(V), $C_{33}H_{54}O_5$, mp 176°, in which one hydroxyl group was left unattacked (the hydroxyl band at 3555 cm^{-1} in Nujol). The nuclear magnetic resonance (NMR) spectrum of IV shows seven C-methyl singlets, one broad signal (*ca.* 5.9 τ) due to a proton geminar to a hydroxyl function and a singlet (3H, 6.32 τ) assignable to a carbomethoxyl group, while that of V indicates the existence of seven methyls and one secondary acetoxyl among wnich the latter is predicted by one proton broad signal centered at *ca.* 4.8 τ ($W_{h/2}$=25 Hz) ascribable to a hydrogen geminar to it. Findings so far suggest that one of eight methyls on a pentacyclic triterpenoid skeleton of III might exist as an oxidized form such as a carboxylic group and two hydroxyl functions would comprise one secondary and one tertiary. As for the tertiary hydroxyl, an isopropanol side chain as encountered in zeorin and leucotylin is inferred on the basis of the dehydration reaction of V with phosphorus oxychloride yielding an isopropenyl (XVI) and isopropylidene (XVII) derivatives as mentioned later.

The gross carbon framework of leucotylic acid (III) has been established as follows. Thus, treatment of methyl leucotylate (IV) with ethanolic hydrogen chloride furnished in a ratio of 1:1 methyl leucotylidienate (VI), $C_{31}H_{48}O_2$, mp 201—203° and an isomer of IV named methyl isoleucotylate (VII), $C_{31}H_{52}O_4$, mp 222°, the structure of which will be discussed later in this paper. The similar result was also observed in case of leucotylin (I), which afforded leucotylidiene along with isoleucotylin.[1)]

The physicochemical properties of VI, especially the IR absorption bands at 785 and 775 cm^{-1}, the ultraviolet (UV) absorption maxima (nm, log ε): 244 (4.29), 252 (4.32), 261 (4.15) of the characteristic heteroannular diene triplet, and the NMR signals at 3.79 and 4.40 τ (2H, AB quartet, J=10.8 Hz) assignable to two cisoid olefinic protons, indicate close resemblance of the diene chromophore of VI to that of 6-O-acetyl-leucotylidiene (XII).[3)] The similarity was also observed in orange coloration of the both dienes by tetranitromethane.

On catalytic hydrogenation over the Adams' catalyst, the diene (VI) was transformed into a monoene mixture (VIII), $C_{31}H_{50}O_2$, which shows no more the absorption maxima due to the diene chromophore in the IR and UV spectra but colors pale yellow by tetranitromethane. Although VIII showed a single spot on thin-layer chromatogram (TLC), it should be a mixture of two monoenes (Δ^{16} and $\Delta^{17(21)}$) as revealed later. Lithium aluminum hydride reduction of VIII produced an alcoholic mixture (IXa), $C_{30}H_{50}O$, whose monoacetyl derivative (IXb), $C_{32}H_{52}O_2$, exhibits the IR absorption bands at 1742 and 1238 cm^{-1} (in Nujol) and an AB quartet signal (2H) at 5.99 and 6.43 τ (J=11 Hz) due to an acetoxymethylene grouping and a diffused signal at *ca.* 4.8 τ (integrated only ½H) ascribable to an olefinic proton (Δ^{16}) in the NMR spectrum. Chromium trioxide oxidation of IXa in acetic acid afforded an aldehydic mixture (X), $C_{30}H_{48}O$, which in turn was submitted to the Huang-Minlon reduction to yield a hydrocarbon mixture (XI) as the colorless leaflets. Each reaction product from VIII through XI has been found by NMR to consist of two double bond position isomers, Δ^{16} and $\Delta^{17(21)}$, which have probably been induced by 1,4 and 1,2 addition of hydrogen to the parent heteroannular diene (VI).

The final hydrocarbon mixture was separated into two components of XIa, mp 180—181° and XIb, mp 153—155° by TLC using silica gel impregnated with silver nitrate. The former compound having lower *Rf* value on TLC was proved to be identical with the authentic sample of hopene-I[10)] by mixed mp, IR (KBr), gas liquid chromatography (GLC) (2% SE-30), and TLC (SiO_2–$AgNO_3$) comparisons, thus putting forward the hopane skeleton as the most probable carbon framework of leucotylic acid except the stereochemistry. The latter one having higher *Rf* value on TLC could be assinged hopene-I isomer, presumably Δ^{16} derivative (XIb), since the NMR spectrum of IXb shows a diffused signal centered at *ca.* 4.8 τ ascribable to an

10) Cordially provided by Dr. S. Natori, National Institute of Hygienic Sciences, Tokyo, to whom the authors' deepest thanks are due.

olefinic proton.

On the other hand, the IR spectrum of methyl leucotylate (IV) in high dilution ($\nu_{max}^{CCl_4}$ cm^{-1} (5×10^{-4}mole) : 3602 (free OH), 3435, 3233 (associated OH)) reveals existence of the intramolecular hydrogen bonding between two hydroxyl functions of IV as was similarly experienced in 6-dehydroleucotylin (XIII).[3]

These accumulated evidences along with consideration on the probable common biogenetic origin of three co-exising triterpenoids: leucotylin(I), zeorin(II), and leucotylic acid(III), have led us to predict leucotylic acid having the hopane skeleton with two hydroxyl functions located at C-16 (secondary) and C-22 (tertiary) as in leucotylin(I).[3] Moreover, two C-methyl singlets at 8.84 and 8.72 τ (3H each) of IV and at 8.84 τ (6H) of V in the NMR spectra assignable to the isopropanol side chain in addition to the above described reaction sequence from III through XIa *via* VI, which is analogous to the leucotylin derivation,[3] are consistent with the prediction.

The hydroxyl function at C-16 of leucotylic acid(III) has been assigned β-equatorial on the basis of (i) easy acetylation of IV, (ii) resistance of IV against chromium trioxide oxidation,

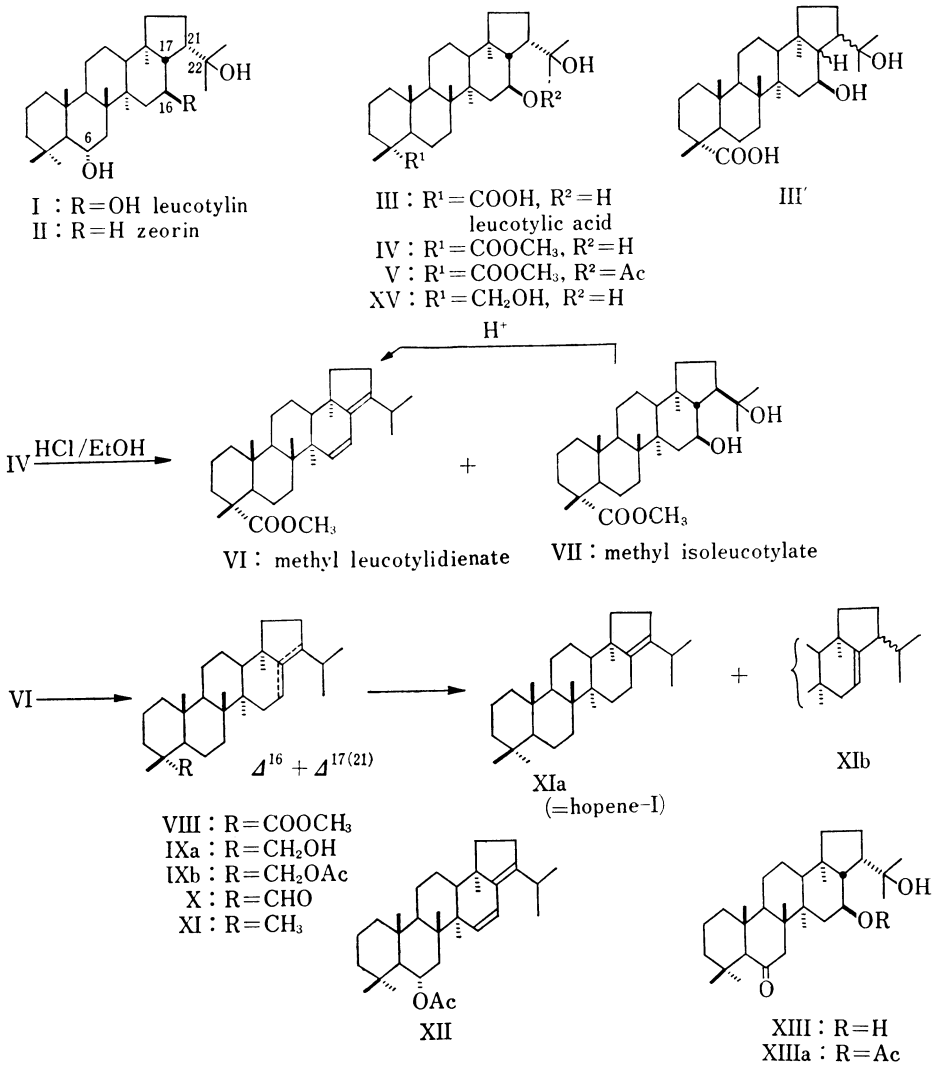


Chart 1

and (iii) the NMR examination which reveals that the proton attached to C-16 in IV or V shows a diffused signal each at *ca.* 5.9 τ (1H, m, $W_{h/2}$=18 Hz) or *ca.* 4.8 τ (1H, m, $W_{h/2}$=25 Hz).[11] In addition, the resembled acetylation increment ($\Delta[M]_D$) for C-16 hydroxyl of leucotylic acid derivatives: Δ $[M]_D$=+293.5° (V−IV) and leucotylin derivatives : $\Delta[M]_D$=+362° (XIIIa−XIII)[3] supports the assignment that both leucotylin(I) and leucotylic acid(III) possess the similar geometry at C-16, *i.e.* C-16β-OH(eq.).

Next, the location of the carboxyl group in leucotylic acid(III) has been elucidated at C-4 with α-equatorial orientation on the basis of the following evidences.

As mentioned briefly above, one of eight methyls on the hopane skeleton is replaced by a carboxyl gruop in leucotylic acid(III). Detailed comparative study on the fragmentation pattern of methyl leucotylate(IV) and leucotylin(I) in the mass spectra has brought out the A or B ring as the possible site of the carbomethoxyl function in IV.

It has hitherto been reported on the mass fragmentation of the hopane triterpenoids that both a-type and b-type fissions occurring on the ring C predominate and the appropriate fragmentations follow.[12] This was the case for both leucotylin(I) and methyl leucotylate (IV) as illustrated in Chart 2 and Chart 3. Comparison of the fragment ions derived from I and IV clearly demonstrates that the carbomethoxyl group of IV locates in the ring A or B. The same fragment ions (*m/e* 223, 205, and 187) probably derived through the a-fission are observed in both compounds, while the fragment ions induced by the b-fission are found at *m/e* 235 (78.3%) and *m/e* 175 (60.9%) in IV and at *m/e* 207 (48.5%) and *m/e* 189 (50%) in I. The similar results were also obtained in the mass spectra of the following two compounds, methyl leucotylidienate (VI) (M^+: *m/e* 452) and 6-O-acetyl-leucotylidiene (XII) (M^+: *m/e* 466). In the former(VI), the fragment ions due to the b-fission are observed at *m/e* 235 (*ca.* 11%) and 175 (*ca.* 13%) with considerable intensity, whereas the latter(XII) does not give rise to an ion at *m/e* 235 but affords a rich ion at *m/e* 189 based on the b-fission. In addition, the ion with *m/e* 187 derived through the a-fission appears as the base peak in both compounds.

Although the several attempts aimed at lactonization or decarboxylation of leucotylic acid(III) and leucotylidienic acid(VI, but COOH instead of $COOCH_3$) were made but without success, the following NMR investigation and the other physical methods have led us to conclude the carboxyl function of III locating at C-4 with α-equatorial orientation.

Thus, on lithium aluminum hydride reduction, methyl leucotylate(IV) afforded a triol(XV), $C_{30}H_{52}O_3$, mp 246—247°, which exhibits the AB quartet signals at 6.44 and 7.04 τ (1H, each, J=10.5 Hz) in its NMR spectrum. Comparison of the methyl signals in the NMR spectra (Table I) of leucotylin(I), zeorin(II), methyl leucotylate(IV) and the triol(XV) reveals that one methyl signal ascribable to a methyl attached to the ring A of IV or XV appears at 8.84[13] or 9.13 τ, while the corresponding methyl at 9.04 or 9.02 τ in I or II respectively. Besides, one methyl signal at 8.86 or 8.83 τ in I or II, which is due to another methyl on the ring A and deshielded by the C-6α hydroxyl function[14] are not seen in IV and XV. These findings suggest that the location of carboxyl group in III is most probably at C-4.

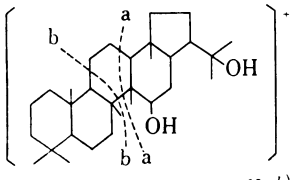
As for the configuration of this carboxyl group, the following evidences are suggestive. As described above the AB quartet signals at 6.44 and 7.04 τ (1H each) of the triol(XV) and at 5.99 and 6.43 τ (1H each) of IXb are assignable to the methylenes of C-4α-CH_2OH and

11) *a*) N.S. Bhacca and D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964, p. 79; b) M. Shamma, R.E. Glick, and R.O. Mumma, *J. Org. Chem.*, **27**, 4512 (1962).

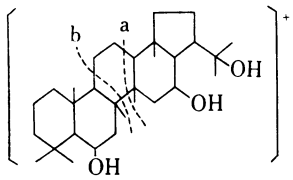
12) *a*) R.E. Corbett and H. Young, *J. Chem. Soc.(c)*, **1966**, 1556 1564; *b*) M.N. Galbraith, C.J. Miller, J.W.L. Rawson, E. Ritchie, J.S. Shannon, and W. C. Taylor, *Aust. J. Chem.*, **18**, 226 (1965); *c*) T. Murakami and C.-M. Chen, *Chem. Pharm. Bull.* (Tokyo), **19**, 25 (1971).

13) Deshielded by a geminar carbomethoxyl group. *cf.* S.W. Pelletier, N. Adityachaudhury, M. Tomasz, J.J. Reynolds, and R. Mechoulam, *Tetrahedron Letters*, **1964**, 3065.

14) S. Huneck and J.-M. Lehn, *Bull. Soc. Chim. France*, **1963**, 1702.



15,22-dihydroxy-hopane [12a,b]



m/e : 460 (M^+), leucotylin (I)

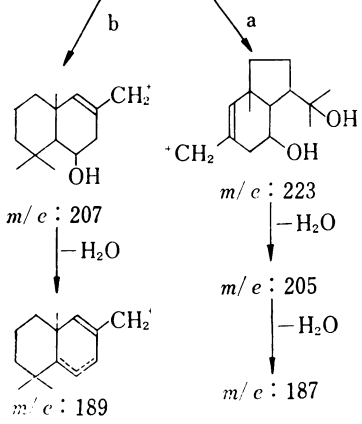


Chart 2. Fragmentation Pattern of Leucotylin (I)

the prominent ion peaks

m/e (%)
460 (2.5) (M^+)
442 (5.8) (M^+-H_2O)
424 (5.8) (M^+-$2H_2O$)
223 (10.7)
207 (48.5)
205 (56.3)
189 (50.0)
187 (31.6)
69 (100.0) (base peak)

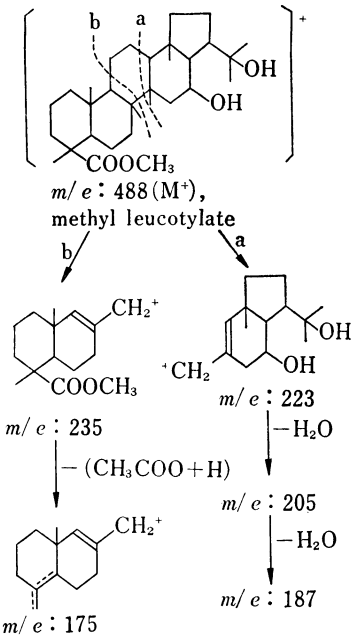


Chart 3: Fragmentation Pattern of Methyl Leucotylate(IV)

the prominent ion peaks

m/e (%)
488 (4.3) (M^+)
470 (7.6)(M^+-H_2O)
452 (6.5) (M^+-$2H_2O$)
428 (2.2) (M^+-CH_3COO-H)
235 (78.3)
223 (23.9)
205 (82.6)
187 (28.3)
175 (60.9)
121 (100.0) (base peak)

-CH_2OAc (both equatorial) respectively,[11b,15] thus supporting the original carboxyl in III as C-4α. Furthermore, the pK_{MCS} value (7.80) of III has been found in good agreement with the calculated value (C-4α-COOH (equatorial): 7.91, C-4β-COOH (axial): 8.41)[16] and a C-O-C stretching absorption band of $COOCH_3$ in IV observed at 1240 cm^{-1} also corroborates the equatorial configuration (*e.g.* equatorial $COOCH_3$: 1245 cm^{-1}, while axial $COOCH_3$: 1150 cm^{-1} and sometimes as doublet.[17]).

Accordingly, the structure of leucotylic acid(III) has been clarified up to III', in which the configurations at C-17 and C-21 are still uncertain and the problem has been dissolved as given below.

15) A. Gaudemer, J. Polonsky, and E. Wenkert, *Bull. Soc. Chim. France*, **1964**, 407.
16) C. Pascual and W. Simon, *Helv. Chim. Acta*, **47**, 683 (1964).
17) S. Bory and M. Fetizon, *Bull. Soc. Chim. France*, **1964**, 570.

TABLE I. The Chemical Shifts of Methyl Signals given in τ Values

	C-4α, C-4β, C-10β (methyls in the ring A)	C-8β	C-14α	C-18α	Geminar methyls at C-22
Leucotylin (I)	8.86,[a] 9.04, 9.17	8.96	8.97	9.25	8.78, 8.86
Zeorin (II)	8.83,[a] 9.02, 9.14	8.96	9.00	9.25	8.83, 8.83
Methyl leucotylate (IV)	— 8.84, 9.14	8.95	9.01	9.22	8.72, 8.84
Triol (XV)	— 9.13, 9.23	8.90	8.98	9.25	8.73, 8.83

a) deshielded by 6α-OH function[14]

Dehydration of methyl 16-O-acetyl-leucotylate (V) with phosphorus oxychloride-pyridine yielded a mixture of two isomers, an isopropylidene derivative(XVII), $C_{33}H_{52}O_4$, mp 178—179.5° and an isopropenyl derivative(XVI), $C_{33}H_{52}O_4$, mp 187.5—189°, in a ratio of 2:1, which were effectively separated by column chromatography using silica gel impregnated with silver nitrate.[18] The respective formulations have been led on the basis of the physical data given in the experimental section. On catalytic hydrogenation[19] over the Adams' catalyst in ethanol, XVI retaining the C-21 configuration of leucotylic acid gave smoothly a saturated compound (XVIII), $C_{33}H_{54}O_4$, mp 160—161°, as a sole product, whose structure has been substantiated by its physical properties: disappearance of the NMR signals and IR absorption bands ascribable to the isopropenyl moiety and regeneration of seven C-methyl signals in the NMR spectra and by negative property for the tetranitromethane test.

On the other hand, similar hydrogenation of XVII in acetic acid-ethyl acetate mixture over the same catalyst furnished a mixture of two saturated isomers, the one identical with XVIII and the other formulated as XIX, $C_{33}H_{54}O_4$, mp 208—210°. The latter could be obtained preferentially in a pure state by repeated recrystallization of the above reaction mixture using ethanol. Both XVIII and XIX should be isomeric at C-21 and could be distinguished each other by GLC, but not by TLC(SiO_2). Since XVIII has been considered to retain the C-21 configuration of III(C-21β-H as revealed later), XIX has been assigned with C-21β oriented isopropyl side chain on the basis of the mechanistic consideration as for the case of leucotylin derivatives.[3]

On alkaline hydrolysis XVIII afforded methyl 22-desoxy-leucotylate(XX), $C_{31}H_{52}O_3$, mp 184.5—186°. Chromium trioxide-pyridine complex oxidation of the latter(XX) gave a ketone(XXI), $C_{31}H_{50}O_3$, mp 174—176°, which was noticed very unstable against acid, alkali, or thermal treatment and hence was transformed preferentially to a stable isomeric ketone (XXII), $C_{31}H_{50}O_3$, mp 195—197.5°.

As has been generally accepted in respect of relative stability between *trans* and *cis* hydrindanones,[20] it has been elucidated among hydroxyhopanone derivatives that the unstable D/E *trans* ketone(XXIII) is readily transformed to the D/E *cis* isomer(XXIV) under acid treatment.[21] In connection with the fact relative stability between *trans* and *cis* hydrindanones possessing the carbonyl function in the six-membered ring has been disclosed similar using leucotylin derivatives. Namely, the diol(XXV) having D/E *trans* ring juncture furnished the *cis* diketone (XXVI) by chromium trioxide-acetic acid oxidation and the *trans* diketone was not detected.[1,3,7] The same was true in the present leucotylic acid derivatives as described above. The 16-keto compound(XXI) (having the α-isopropyl function at C-21 as revealed

18) T. Norin and L. Westfelt, *Acta Chem. Scand.*, **17**, 1828 (1963).
19) I. Yosioka, T. Nakanishi, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 5185 (Preliminary account on the catalytic hydrogenation of the dehydration product.).
20) D.H.R. Barton and G.A. Morrison, "Fortschritte der Chemie Organischer Naturstoffe," Vol. 19, ed. by L. Zechmeister, Springer-Verlag, Vienna, **1961**, p. 179.
21) *a*) H. Fazackerley, T.G. Halsall, and E.R.H. Jones, *J. Chem. Soc.*, **1959**, 1877; *b*) G.U. Baddeley, T.G. Halsall, and E.R.H. Jones, *J. Chem. Soc.*, **1960**, 1715.

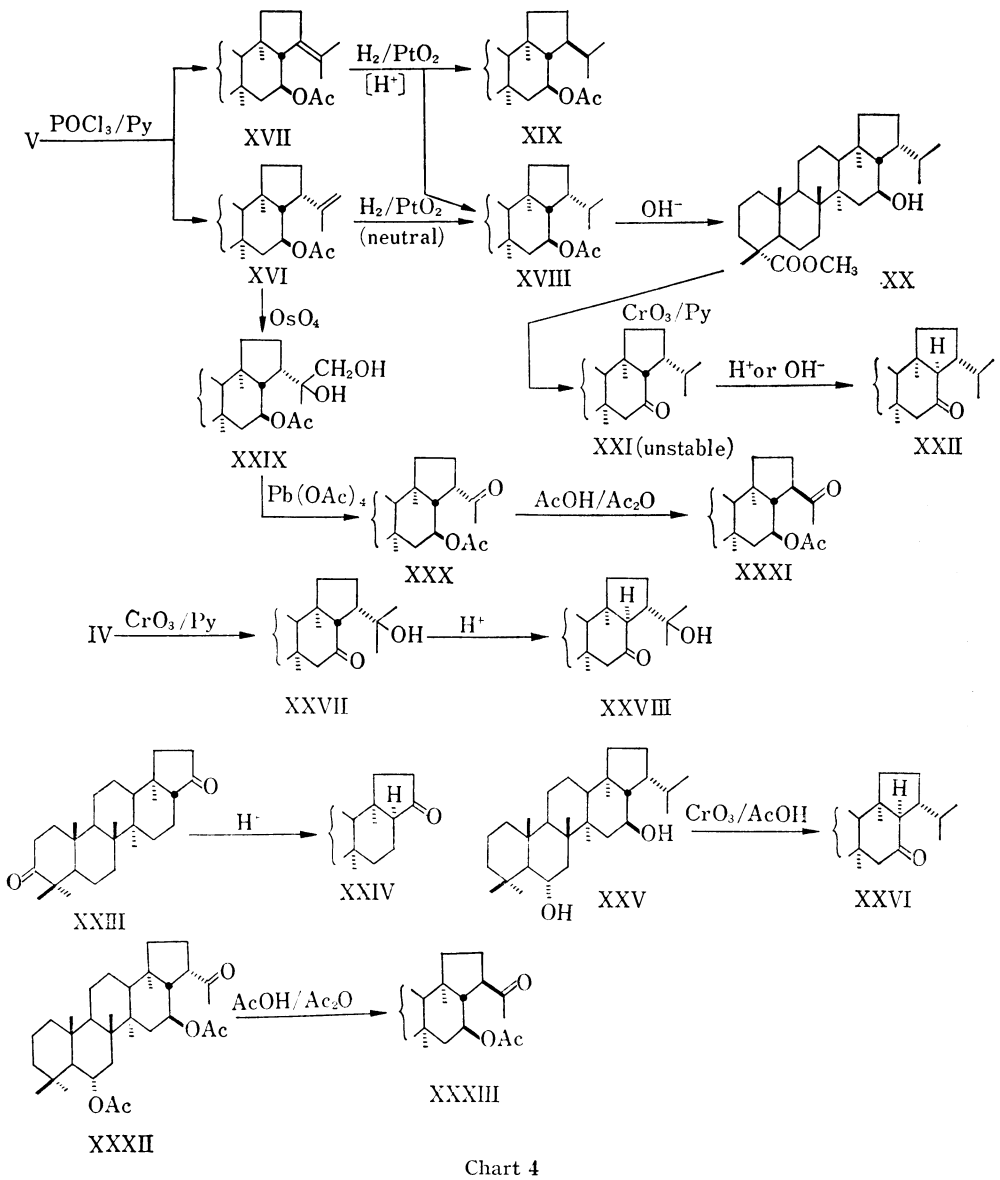


Chart 4

later) was inverted to the isomer(XXII), thus suggesting XXI to be unstable *trans* while XXII *cis*. Similarly, chromium trioxide-pyridine oxidation of methyl leucotylate(IV) furnished a D/E *trans* ketone(XXVII), $C_{31}H_{50}O_4$, mp 190°, which was readily isomerized to a *cis* ketone(XXVIII), mp 208°, under acid treatment.

Therefore, leucotylic acid(III) has been clarified to carry C-17β-H and the C-21 geometry left as the final problem on the structure of III has been made clear as below.

The isopropenyl derivative(XVI) was submitted to osmium tetroxide oxidation followed by hydrogen sulfide treatment to yield a glycol(XXIX), $C_{33}H_{54}O_6$, mp 219—221°. On subsequent treatment with lead tetraacetate in anhydrous benzene, XXIX produced an unstable methylketone(XXX), $C_{32}H_{50}O_5$, mp 158—160°, which was then transformed to a stable isomer(XXXI), $C_{32}H_{50}O_5$, mp 185.5—187° under reflux in acetic acid-acetic anhydride

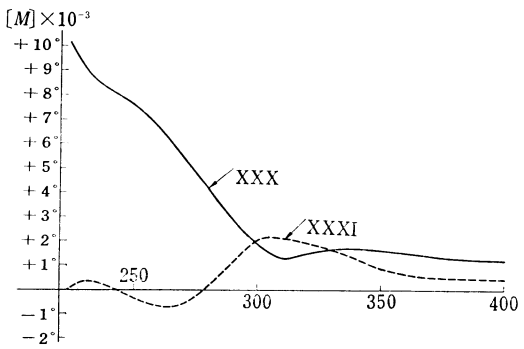


Fig. 1. The ORD Curves of XXX and XXXI in MeOH

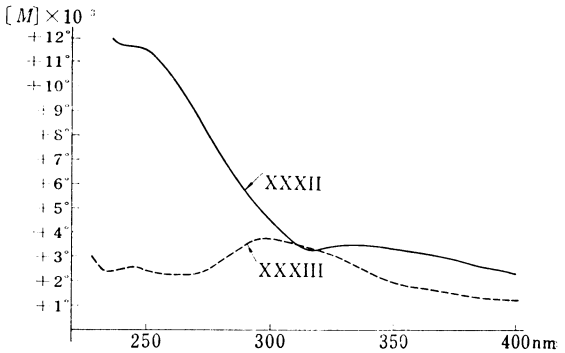


Fig. 2. The ORD Curves of XXXII and XXXIII in MeOH[1)]

mixture. The optical rotatory dispersion(ORD) examination of both methylketones, XXX and XXXI, in comparison with leucotylin derivatives, XXXII and XXXIII,[1)] distinctly demonstrates that XXX and XXXII possess the similar chirality around the carbonyl environement whereas XXXI and XXXIII do similarly (Fig. 1 and Fig.2). The unstable behavior of XXX is analogously explained as for hydroxyhopanone derivatives,[22)] adiantone,[23)] leucotylin and zeorin derivatives.[1,7)]

Consequently, XXX and hence leucotylic acid should be expressed as having C-21β-H, thus finally concluding the total structure of the latter as III.

The Structure of Methyl Isoleucotylate(VII)[9)]

As described before, reflux of methyl leucotylate(IV) in ethanolic hydrogen chloride yielded an isomer named methyl isoleucotylate(VII) together with the diene(VI). Methyl isoleucotylate(VII), $C_{31}H_{52}O_4$, mp 222°, $[\alpha]_D$ +40° ($CHCl_3$), possesses the same molecular formula and behavior on TLC as methyl leucotylate(IV). It showed negative for tetranitromethane. On acetylation with acetic anhydride–pyridine, VII gave methyl 16-O-acetylisoleucotylate(XXXIV), $C_{33}H_{54}O_5$, mp 220°, in which one hydroxyl function was left unattacked as shown by the IR absorption band at 3550 cm^{-1} (in $CHCl_3$). Chromium trioxide oxidation of methyl isoleucotylate(VII) in pyridine afforded a monoketone(XXXV), $C_{31}H_{50}O_4$, mp 212°, in which one hydroxyl function was also unaffected (3400 cm^{-1} in $CHCl_3$) and another hydroxyl was converted to a carbonyl (1695 cm^{-1}). The ketone was found stable for either acid or alkali, suggesting the ketone to be a stable form.

The examination of the IR and NMR data of VII, XXXIV, and XXXV indicates the existence of two hydroxyl functions, *i.e.* one secondary and one tertiary, seven C-methyl groups, and one carbomethoxyl function in VII. Especially of significance is that two C-methyl signals observed around 8.78—8.84 τ rationalize the partial constitution including the tertiary hydroxyl to be an isopropanol group as in IV. The existence of an isopropanol function in VII was endorsed by preparing an isopropenyl derivative(XXXVII) from XXXIV by dehydration with phosphorus oxychloride as mentioned later. Moreover, the remarkable resemblance of the NMR and IR spectra between VII and IV has led us to assume the similar carbon skeleton for both isomers, which has now been verified by the evidence below.

Prolonged acid treatment of VII afforded the diene(VI) as IV did. The fact is reminiscent of the disposition of two hydroxyl functions in VII possibly at C-16 and C-22 as in IV. The intramolecular hydrogen bonding(IR $\nu_{max}^{CCl_4}$ cm^{-1} (5×10^{-4} mole): 3603 (free), 3434, 3222(bonded OH)) ascribable to C-16 and C-22 hydroxyls in VII supports the assumption. The configura-

22) G.U. Baddeley, T.G. Halsall, and E.R.H. Jones, *J. Chem. Soc.*, **1961**, 3891.
23) G. Berti, F. Bottari, A. Marsili, J.-M. Lehn, R. Witz, and G. Ourisson, *Tetrahedron Letters*, **1963**, 1283.

tion of hydroxyl group at C-16 is considered as retained β-equatorial since the NMR spectra of VII and XXXIV show the diffused signals at *ca.* 6.0 τ (1H, m, $W_{h/2}$=18 Hz) and *ca.* 5.1 τ (1H, m, $W_{h/2}$=*ca.* 27 Hz) respectively.[11]

It follows therefore that the possible difference of IV and VII could be ascribed to the configuration of either/both at C-17 or/and at C-21.

Since the stable monoketone(XXXV) derived from VII was identical neither with XXVII nor XXVIII, the configurational difference at C-21 has become more likely (*i.e.* C-21α-H in VII). Among two possibilities for XXXV, one with C-17α-H and C-21α-H and another with C-17β-H and C-21α-H, the latter seems more probable. In the former the severe interaction between C-21 side chain and C-15 methylene is anticipated and this makes the formulation less likely and moreover guarantees the stability of XXXV if *trans* hydrindanone. This was the case in 6,16-diketo-22-desoxy-21αH-leucotylin(XXXVI) as presented previously.[1] The fact that the ORD curve of XXXV is quite resembled to that of XXXVI rather than to that of XXII(C-17α-H, C-21β-H) is consistent with the assignment. Furthermore, the NMR examination of iso derivatives(VII and XXXIV) in comparison with methyl leucotylate derivatives (IV and V) has corroborated additionally. Thus, both the methyl signals appearing at the highest field and assigned to C-18α methyl and the diffused signals due to C-16α-H are observed at higher field in iso derivatives(VII: 9.25, *ca.* 6.0 τ; XXXIV: 9.18, *ca.* 5.1 τ) than in methyl leucotylate derivatives (IV: 9.22, *ca.* 5.9 τ; V: 9.14, *ca.* 4.8 τ) respectively as experienced in leucotylin derivatives (6,16-di-O-acetyl-isoleucotylin: 9.21, *ca.* 5.0 τ and 6,16-di-O-acetyl-leucotylin: 9.16, *ca.* 4.8 τ).[1]

Finally, the inferrence leading to the structure (VII) for methyl isoleucotylate has been substantiated by the following derivations. Treatment of methyl 16-O-acetyl-isoleucotylate (XXXIV) with phosphorus oxychloride in pyridine afforded an isopropenyl derivative (XXXVII), $C_{33}H_{52}O_4$, mp 221° as a single product, and the formulation was agreed with the physical data. This is the significant evidence since methyl 16-O-acetyl-leucotylate (V) and 6,16-di-O-acetyl-leucotylin both carrying the C-17β-H and C-21β-H configuration furnished

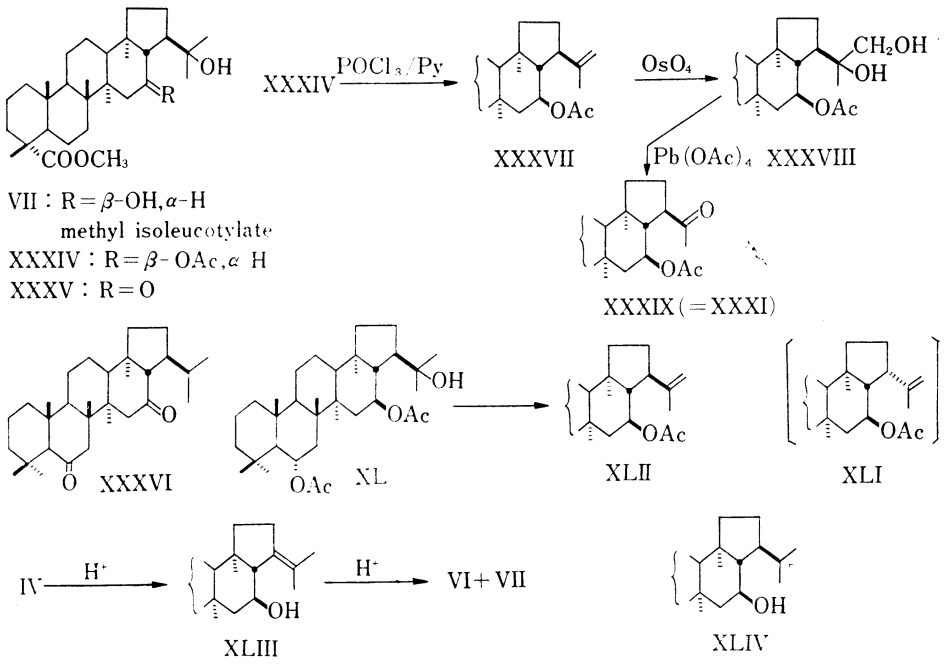


Chart 5

Table II. Comparison of the Physical Data of Isopropenyl Derivatives having C-21β-H and C-21α-H Configurations

		Leucotylic acid derivatives		Leucotylin derivatives[1]	
		XVI (C-21β-H)	XXXVII (C-21α-H)	XLI (C-21β-H)	XLII (C-21α-H)
IR ($CHCl_3$) cm^{-1}:	$>C=CH_2$	1623, 1648(sh), 892	1640, 890	1630(br), 890	1642, 887
NMR ($CDCl_3$) τ:	$>C_{(18)}$-CH_3	9.11	9.17	9.14	9.18
	$>C_{(16)}H$-$OCOCH_3$	8.03	8.14	8.06	8.12
	$>C=CH_2$	5.30	5.37	5.29	5.36

the mixture of the isopropenyl and isopropylidene derivatives respectively, while 6,16-di-O-acetyl-isoleucotylin (XL) afforded the isopropenyl compound (XLII) as the sole product.[1]

Comparison of the physical properties of XVI and XXXVII with those of two isopropenyl derivatives of leucotylin (Table II), has suggested that XXXVII bears the C-21β isopropenyl moiety as XLII. The isopropenyl compound (XXXVII) was then submitted to osmium tetroxide oxidation followed by hydrogen sulfide treatment giving a glycol (XXXVIII), $C_{33}H_{54}O_6$, mp 200°, which in turn was oxidized with lead tetraacetate in anhydrous benzene to yield a methylketone (XXXIX), $C_{32}H_{50}O_5$, mp 183.5—185°. These procedures assure the retention of C-21 configuration from XXXIV to XXXIX. Since XXXIX was stable against both acid and alkali, XXXIX could be assigned C-21α-H type similarly as before.[1,22,23] Importantly, XXXIX thus obtained was found identical by means of mixed mp, IR ($CHCl_3$), ORD (MeOH), and TLC with the aforementioned stable methylketone(XXXI) which was derived from methyl leucotylate (IV) *via* XVI and XXX and possesses the C-21α-H configuration.

Consequently, methyl isoleucotylate is now assigned VII having the C-21β isopropanol moiety, namely the reversed configuration at C-21 as compared with methyl leucotylate (IV).

It should be pointed out here again that the isopropanol moieties in methyl 16-O-acetyl-leucotylate (V) and methyl 16-O-acetyl-isoleucotylate (XXXIV) show distinctly different behavior for dehydration. Thus, V produced a mixture of XVI and XVII (with the ratio of 1:2) while XXXIV gave XXXVII predominantly. As for the reason, the 1,3-steric interaction between C-18α methyl and C-21α side chain in V is considered remarkable and possibly responsible for major dehydration path of V towards XVII, thus releasing the interaction. These features have been found among other hopane derivatives: hydroxyhopane,[24] leucotylin (I)[3] and zeorin (II).[1,5,7] In case of XXXIV, such congestion does not exist so that dehydration proceeds preferentially to XXXVII as have been demonstrated in the isohopane derivatives.[3,24]

Furthermore, in order to clarify the mode of isomerization at C-21 starting from methyl leucotylate (IV) to methyl isoleucotylate (VII), further investigation along this line has been undertaken. On mild acid treatment for 5 minutes, IV yielded an isopropylidene derivative (XLIII), $C_{31}H_{50}O_3$, mp 183°, which on further acid treatment was transformed into a mixture of the diene (VI) and methyl isoleucotylate (VII) with a ratio of 1:1. The evidence would make it probable that XLIII is an intermediate from IV to VI and VII. Namely, at the initiation XLIII is formed by dehydration of IV, then VI or VII is produced either by further dehydration followed by double bond migration or hydration *via* a probable intermediate (XLIV) giving a more stable C-21α-H skeleton. Although such a hydration procedure has never been found in the hopane derivatives, it has been presumed that the C-16β hydroxyl function in I or IV might be responsible for furnishing the iso derivative under acid treatment.

Since our preliminary account on leucotylic acid (III) was presented,[8] some acidic tri-

24) Y. Tsuda, K. Isobe, S. Fukushima, H. Ageta, and K. Iwata, *Tetrahedron Letters*, **1967**, 23.

terpenoids having the hopane skeleton have been isolated in Nature. They are pyxinic acid,[25] phlebic acids A[26] and B[27] from the lichen and woodwardic acid from the fern.[12c]

Experimental[28]

Isolation of Leucotylic Acid (III)——During the successive ether extraction of the air dried lichen, *Parmelia leucotyliza* Nyl. (4 kg) collected at Sugio in Osaka-fu, a crop of crystals (atranorin, recrystallized with acetone) was precipitated. The combined ether soluble parts were treated with aqueous 5% $NaHCO_3$ and 5% NaOH successively as described previously.[3] The combined alkali soluble part containing the precipitates of sodium leucotylate was neutralized with aqueous 10% HCl and treated in a usual manner to give a solid (26.7 g). A part of the solid (8.2 g), after washing with ether in order to remove the colored resinous component, was chromatographed on silica gel column eluting with $CHCl_3$, $CHCl_3$–AcOEt (4:1—1:1) successively. The eluate with $CHCl_3$ yielded atranorin (recrystallized with acetone). The product obtained by elution with $CHCl_3$–AcOEt mixture (4:1 and 1:1) was washed with petr. ether and ether and then recrystallized with MeOH to afford leucotylic acid (III) (colorless plates, 1.35 g), mp 259—260°. $[\alpha]_D$ +330° (c=0.15, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.95; H, 10.82 and 76.11; H, 10.82. IR ν_{max}^{Nujol} cm^{-1}: 3380 (sh), 3200 (OH), 1710 (sh), 1690 (COOH). By repeating the same procedure twice, leucotylic acid (1.8 g and 1.5 g each) was obtained from the above solid (10 g and 8.5 g respectively). The total yield of leucotylic acid was 4.65 g (0.12%) from the air dried lichen (4 kg).

Methyl Leucotylate (IV)——To a solution of leucotylic acid (III) (1.3 g) in EtOH (250 ml), was added ethereal diazomethane and the solution was let stand at about 5° overnight. The crude crystals obtained by evaporation of the solvent were recrystallized with EtOH to give methyl leucotylate (IV) (colorless plates, 1.1 g), mp 298—301°, $[\alpha]_D$ +29° (c=2, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72. Found: C, 76.34; H, 10.74 and C, 76.44; H, 10.66. Mass Spectrum m/e: 488 (M^+). IR ν_{max}^{Nujol} cm^{-1}: 3200 (OH), 1720, 1240 ($COOCH_3$). NMR ($CDCl_3$) τ: 9.22, 9.14, 9.01, 8.95, 8.72 (3H, each, s), 8.84 (6H, s) (totally $7\times CH_3$), 6.32 (3H, s, $COOCH_3$), *ca.* 5.9 (1H, m, $W_{h/2}$=18 Hz, $>C_{(16)}H$-OH).

Methyl 16-O-Acetyl-leucotylate (V)——Methyl leucotylate (IV) (0.6 g) was treated with pyridine (20 ml and Ac_2O (8 ml) at room temperature overnight. The crude product obtained after the usual manner was purified on neutral alumina (Woelm) column eluting with benzene. The product (0.6 g) was then recrystallized with aqueous MeOH to yield the acetate (V) (colorless needles), mp 116°, $[\alpha]_D$ +95° (c=0.95, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_5\cdot\frac{1}{4}H_2O$: C, 73.98; H, 10.17. Found: C, 74.05; H, 10.21. IR ν_{max}^{Nujol} cm^{-1}: 3555 (OH), 1755 ($OCOCH_3$), 1730 ($COOCH_3$), 1240; $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530 (OH), 1740 ($OCOCH_3$), 1715 ($COOCH_3$). NMR ($CDCl_3$) τ: 9.14 (6H, s), 9.02 (3H, s), 8.84 (12H, s) (totally $7\times CH_3$), 7.94 (3H, s, $OCOCH_3$), 6.36 (3H, s, $COOCH_3$), *ca.* 4.8 (1H, m, $W_{h/2}$=25 Hz, $>C_{(16)}H$-OAc). The acetate (V) gave colorless needles of mp 176° on recrystallization with *n*-hexane. *Anal.* Calcd. for $C_{33}H_{54}O_5$: C, 74.67; H, 10.26. Found: C, 74.61; H, 10.33.

Acid Treatment of Methyl Leucotylate (IV) giving Methyl Leucotylidienate (VI) and Methyl Isoleucotylate (VII)——A solution of methyl leucotylate (IV) (1 g) in conc. HCl (33 ml)–EtOH (170 ml) mixture was refluxed for 30 min and diluted with water. The precipitates were collected, washed with water, dried, and purified by column chromatography using neutral alumina (Woelm). The product obtained by eluting with benzene was recrystallized with EtOH to give the diene (VI) as colorless needles (435 mg) of mp 201—203°. *Anal.* Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found: C, 82.19; H, 10.49. Mass Spectrum m/e: 452 (M^+). IR ν_{max}^{Nujol} cm^{-1}: 1720, 1240 ($COOCH_3$), 785, 775. UV λ_{max}^{EtOH} nm (log ε): 244 (4.29), 252 (4.32), 261 (4.15). NMR ($CDCl_3$) τ: 9.11 (6H, s), 9.07 (3H, s), 9.01 (3H, d, J=7.2 Hz), 8.98 (3H, d, J=7.2 Hz), 8.80 (6H, s) (totally $7\times CH_3$), 6.29 (3H, s, $COOCH_3$), 3.79, 4.40 (2H, AB q, J=10.8 Hz, $-C_{(15)}H{=}C_{(16)}H-$).

The product obtained by $CHCl_3$ elution was recrystallized with EtOH to give methyl isoleucotylate (VII) (colorless needles, 410 mg), mp 222°, $[\alpha]_D$ +40° (c=2, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72. Found: C, 76.22; H, 10.60. IR ν_{max}^{Nujol} cm^{-1}: 3250 (OH, broader band than that of IV), 1730, 1240 ($COOCH_3$); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (OH). NMR ($CDCl_3$) τ: 9.25 (3H, s), 9.13 (3H, s), 8.99 (3H, s), 8.97 (3H, s), 8.83 (6H, s), 8.78 (3H, s) (totally $7\times CH_3$), 6.32 (3H, s, $COOCH_3$), *ca.* 6.0 (1H, m, $W^{h}/_{2}$=18 Hz, $>C_{(16)}H$-OH). VII showed the same Rf value as methyl leucotylate (IV) on TLC.

Catalytic Hydrogenation of Methyl Leucotylidienate (VI) giving Monoene (VIII)——The diene (VI) (860 mg) was hydrogenated over PtO_2 (200 mg) in AcOH (100 ml)–AcOEt (100 ml) mixture at room temperature

25) I. Yosioka, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 613.
26) R. Takahashi, O. Tanaka, and S. Shibata, *Phytochemistry*, **8**, 2345 (1969).
27) R. Takahashi, O. Tanaka, and S. Shibata, *Phytochemistry*, **9**, 2037 (1970).
28) Melting points were taken on the Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and recorded as read. The following instruments were used: Rex Photoelectric Polarimeter NEP-2 for $[\alpha]_D$, Yanagimoto Gas Chromatograph Model GCG-3DH with FID for GLC, Hitachi EPI-2 or EPI-S2 Spectrometer for IR, Varian A-60 and Hitachi H-60 Spectrometer for NMR, Hitachi RMU-6D Mass Spectrometer fo Mass spectra, and JASCO ORD/UV-5 Automatic Recording Spectropolarimeter for ORD.

for 4.5 hr until one mole of hydrogen was uptaken. After the usual work-up the product was recrystallized with EtOH to give a monoene mixture (VIII) as colorless crystals (850 mg) mp 208—209°. *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.81: H, 11.27. IR ν_{max}^{Nujol} cm^{-1}: 1720, 1240 ($COOCH_3$). UV (EtOH): only end absorption.

$LiAlH_4$ Reduction of VIII giving Alcohol (IXa)——To a solution of VIII (825 mg) in anhydrous ether (150 ml), was added $LiAlH_4$ (1.5 g) and the total mixture was stirred for 6.5 hr at room temperature and let stand two days. After successive treatment with water (1.5 ml), aq. 1.5% NaOH (1.5 ml) and water (5 ml), the reaction mixture was filtered and the filtrate was washed with water and treated as usual. Evaporation of the solvent followed by recrystallization with MeOH gave an alcohol mixture (IXa) (colorless needles, 730 mg), mp 168.5—170.5°. *Anal.* Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.64; H, 11.79. IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH).

Acetylation of IXa giving IXb——IXa (120 mg) was treated with pyridine (5 ml) and Ac_2O (2 ml) at room temperature overnight. After usual treatment, the product was recrystallized with EtOH to yield IXb (colorless needles, 100 mg), mp 140.5—141°. *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.97; H, 11.28. IR ν_{max}^{Nujol} cm^{-1}: 1742, 1238 ($OCOCH_3$). NMR ($CDCl_3$) τ: 5.99, 6.43 (1H, each, AB q, $J=11$ Hz, $>C_{(4)}$-CH_2OAc), *ca.* 4.8 (*ca.* 1/2H, m, olefinic proton).

Oxidation of IXa giving Aldehyde (X)——To a solution of IXa (580 mg) in AcOH (150 ml), was added a mixture of CrO_3 (96 mg) in 95% AcOH (40 ml) under ice cooling and the total mixture was stirred for 2 hr below 15°. The product obtained after the usual way was chromatographed on silica gel column eluting with *n*-hexane–benzene (4:1—3:1). Recrystallization of the product with EtOH yielded an aldehydic mixture (X) as colorless plates (200 mg), mp 157—158°. *Anal.* Calcd. for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.61; H, 11.34. IR ν_{max}^{Nujol} cm^{-1}: 1724, 2646 (CHO).

Huang Minlon Reduction of X giving Two Hydrocarbons, XIa(=Hopene-I) and XIb——A solution of X (130 mg) in triethyleneglycol (35 ml) was added with 80% hydrazine hydrate (3.5 ml) and KOH (5 g) and refluxed in an oil bath (bath temp. 165—170°) for 3.5 hr. After setting the condenser downward, the bath temperature was raised up to 240° gradually to distill out unreacted hydrazine and water completely. The downward condenser was then replaced by an air-cooler and the mixture was heated in the oil bath (235—240°) for further 3.5 hr. When the reaction mixture was poured into ice water, the colorless crystals were precipitated. The crystalline product was then purified on silica gel column and recrystallized with acetone to give a hydrocarbon mixture (XI). Although the hydrocarbon (XI) showed a single spot on TLC (SiO_2), it was revealed by means of TLC (SiO_2–$AgNO_3$) and GLC (2% SE-30, column temp. 248°) that the hydrocarbon consisted of two isomers (Δ^{16} and $\Delta^{17(21)}$) with a ratio of 13:12. Therefore, the hydrocarbon mixture was separated by preparative TLC (SiO_2–$AgNO_3$) developing with *n*-hexane to give two hydrocarbons, namely XIa (*Rf* vale: 0.17) and IXb (*Rf* value: 0.27) respectively. The slow moving hydrocarbon was recrystallized with acetone to give pure XIa, mp 180—181°, which was identified with authentic hopene-I (mp 182—183°)[10] by mixed mp, IR (KBr), TLC (SiO_2–$AgNO_3$), and GLC (2% SE-30). Another hydrocarbon was recrystallized with acetone to yield colorless needles, mp 153—155°, which is presumably formulated as XIb, since the olefinic proton is observed at 4.8 τ (1H, m) in the NMR spectrum of IXb.

$LiAlH_4$ Reduction of Methyl Leucotylate (IV) giving Triol (XV)——To a solution of methyl leucotylate (IV) (130 mg) in anhydrous ether (150 ml), was added $LiAlH_4$ (700 mg) and the mixture was stirred at room temperature for 8 hr. After successive treatment with water (0.7 ml), aq. 15% NaOH (1 ml) and water (2 ml) followed by filtration to remove the precipitates, the filtrate was washed with water and treated in the usual manner. Recrystallization of the product with acetone yielded the triol (XV) as colorless plates (100 mg), mp 246—247°. *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found: C, 78.49; H, 11.20. IR ν_{max}^{Nujol} cm^{-1}: 3440, 3540 (w) (OH). NMR ($CDCl_3$) τ: 9.25, 9.23, 9.13, 8.98, 8.90, 8.83, 8.73 (3H, each, all s, totally $7\times CH_3$), 6.44, 7.04 (1H, each, AB q, $J=10.5$ Hz, $>C_{(4)}$-CH_2OH), *ca.* 6.0 (1H, m, $>C_{(16)}H$-OH).

Measurement of pK_{MCS} of Leucotylic Acid (III)——A solution of leucotylic acid (III) in methyl cellosolve-water (80:20 w/w) mixture was titrated with aqueous 0.1 N NaOH under stirring. The pK_{MCS} value (=7.8) was obtained from the calibration curve.

Dehydration of Methyl 16-O-Acetyl-leucotylate (V) with $POCl_3$ yielding Isopropenyl (XVI) and Isopropylidene (XVII) Derivatives——A solution of methyl 16-O-acetyl-leucotylate (V) (2.1 g) in pyridine (50 ml) was treated with $POCl_3$ (8 ml) at room temperature overnight. Since the product obtained after usual treatment comprised two isomers as revealed by TLC (SiO_2–$AgNO_3$), the mixture was chromatographed on SiO_2–$AgNO_3$ column[18] developing with *n*-hexane–benzene mixture (2:1—1:1) successively. The earlier eluate (950 mg) with *n*-hexane–benzene (2:1) was recrystallized with MeOH to yield the isopropylidene compound (XVII) as colorless plates, mp 178—179.5°. *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.55; H, 10.27 and C, 77.46; H, 10.00. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1728, 1244 ($OCOCH_3$). NMR ($CDCl_3$) τ: 9.33, 9.11, 9.00, 8.87, 8.82 (3H, each, all s, $5\times CH_3$), 8.37 (6H, s, $>C=C(CH_3)_2$), 7.94 (3H, s, $OCOCH_3$), 6.32 (3H, s, $COOCH_3$), *ca.* 4.8 (1H, m, $>C(s)_{16}(s)H$-OAc). The later eluate with *n*-hexane–benzene (2:1) and the eluate with *n*-hexane–benzene (1:1) gave a product (450 mg) which was recrystallized with MeOH to afford the isopropenyl compound (XVI) as colorless needles, mp 187.5—189°. *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.29; H, 10.35. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1727, 1243 ($OCOCH_3$), 1648, 1623, 892 ($>C=CH_2$). NMR

($CDCl_3$) τ: 9.11, 9.08, 8.99 (3H, each, s), 8.82 (6H, s) (totally $5\times CH_3$), 8.26 (3H, s, CH_3-$\dot{C}$=CH_2), 5.30 (2H, s, >C=CH_2), 8.03 (3H, s, $OCOCH_3$), 6.34 (3H, s, $COOCH_3$), *ca.* 4.8 (1H, m, $>C_{(16)}H$-OAc).

Hydrogenation of Isopropenyl Derivative (XVI) giving Methyl 16-O-Acetyl-22-desoxy-leucotylate (XVIII)——The isopropenyl compound (XVI) (60 mg) in EtOH (30 ml) was hydrogenated over PtO_2 (60 mg) under stirring at room temperature for 3 days and treated in a usual manner. In fact, one hour was enough for uptake of 1 mole of hydrogen. The product was then recrystallized with aqueous EtOH to yield a saturated compound (XVIII) as colorless needles (50 mg), mp 160—161°. *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.59. Found: C, 76.60; H, 10.68. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1718, 1252 ($OCOCH_3$). NMR ($CDCl_3$) τ: 8.81—9.15 (totally $7\times CH_3$), 7.95 (3H, s, $OCOCH_3$), 6.31 (3H, s, $COOCH_3$), *ca.* 4.9 (1H, m, $>C_{(16)}H$-OAc). XVIII gave only the peaks due to the degradation products on GLC (1% SE-30, column temp. 240°) and was negative for tetranitromethane. In the same manner, the isopropenyl derivative (XVI) (1.22 g) in EtOH (60 ml) was hydrogenated over PtO_2 (370 mg) at room temperature for 4 hr to yield XVIII (1.05 g).

Hydrogenation of Isopropylidene Derivative (XVII) giving XVIII and Methyl 16-O-Acetyl-22-desoxy-21αH-leucotylate (XIX)——The isopropylidene derivative (XVII) (250 mg) was hydrogenated over PtO_2 (70 mg) in AcOH (20 ml)–AcOEt (10 ml) mixture under stirring at room temperature for 14 hr. Although the product showed a single spot on TLC (Silica gel G), it gave the degradation peaks derived from XVIII and a peak of XIX on GLC (1% SE-30, column temp. 240°). Recrystallization of the product with EtOH furnished pure XIX as colorless needles, mp 208—210°. *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.59. Found: C, 77.25; H, 10.66. IR ν_{max}^{KBr} cm^{-1}: 1724, 1245. NMR ($CDCl_3$) τ: 8.82—9.20 (totally $7\times CH_3$), 7.99 (3H, s, $OCOCH_3$), 6.33 (3H, s, $COOCH_3$), *ca.* 5.0 (1H, m, $>C_{(16)}H$-OAc).

Alkaline Hydrolysis of XVIII giving Methyl 22-Desoxy-leucotylate (XX)——XVIII (847 mg) was treated with 5% KOH–EtOH (800 ml) under reflux on a water-bath for 30 min. The crude product (765 mg) was recrystallized with MeOH to give XX of melting at 184.5—186°. *Anal.* Calcd. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.71; H, 10.96. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3450 (OH).

Oxidation of XX yielding Methyl 16-Dehydro-22-desoxy-leucotylate (XXI)——XX (465 mg) was treated with CrO_3–pyridine complex with stirring at room temperature for 3 hr as usual. The crude product containing the strating material (XX) (recovered yield, 226 mg) was purified by preparative TLC (SiO_2) developing with benzene followed by recrystallization with aqueous MeOH to give the ketone (XXI) (colorless crystals, 190 mg), mp 174—176°. *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: 79.36; H, 10.66. IR ν_{max}^{KBr} cm^{-1}: 1733, 1233 ($OCOCH_3$), 1725 (CO). NMR ($CDCl_3$) τ: 8.91—9.28 (totally $7\times CH_3$), 6.41 (3H, s, $COOCH_3$). ORD (c=0.17, dioxane) $[\alpha]^{10°}$ (nm): +12° (589), +24° (450), 0° (350), −168° (322) (trough), 0° (308), +222° (300), +383° (290), +431° (278) (peak), +419° (273), +371° (266).

Isomerization of XXI to Methyl 16-Dehydro-22-desoxy-17αH-leucotylate (XXII)——Treatment of XXI either with 5% KOH–EtOH or with 5% HCl–EtOH under reflux for 10 min yielded the isomer (XXII) preferentially. Recrystallization with aqueous EtOH gave pure XXII, mp 195—197.5°. *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 78.77; H, 10.56. IR ν_{max}^{KBr} cm^{-1}: 1732, 1250 ($COOCH_3$), 1716 (CO). NMR ($CDCl_3$) τ: 8.90—9.11 (totally $7\times CH_3$), 6.41 (3H, s, $COOCH_3$). ORD (c=0.11, MeOH) $[\alpha]^{9°}$ (nm): −9° (589), −309° (350), −1163° (320), −1545° (309) (trough), 0° (296), +2363° (270) (peak), +1909° (250). XXII was also obtained by heating XXI at 180°.

Oxidation of Methyl Leucotylate (IV) giving Methyl 16-Dehydro-leucotylate (XXVII) and Isomerization of XXVII——Methyl leucotylate (IV) (1 g) was treated with CrO_3 (1 g)–pyridine (30 ml) complex with stirring for 42 hr. The crude product obtained after usual treatment was purified by neutral alumina column chromatography eluting with benzene and recrystallized with aqueous EtOH to give XXVII (colorless needles, 50 mg), mp 190°. *Anal.* Calcd. for $C_{31}H_{50}O_4\cdot\frac{1}{4}H_2O$: C, 75.81; H, 10.36. Found: C, 75.91; H, 9.88. Mass Spectrum m/e: 486 (M^+ of $C_{31}H_{50}O_4$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (OH), 1710 ($COOCH_3$), 1650 (CO). NMR ($CDCl_3$) τ: 9.10, 8.99, 8.97, 8.90, 8.85, 8.74, 8.65 (3H, each, all s, $7\times CH_3$), 6.35 (3H, s, $COOCH_3$). ORD (c=0.8, MeOH) $[\alpha]$ (nm): +125° (589), +302° (400), 0° (352), −30° (346) (trough), 0° (340), +557° (317) (peak), +388° (310).

A solution of XXVII (15 mg) in 5% HCl–MeOH (3 ml) was refluxed for 15 min and the product obtained after the usual manner was purified by silica gel column chromatography developing with benzene followed by recrystallization with n-hexane to give XXVIII (9 mg), mp 208°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (OH), 1710 ($COOCH_3$), 1695 (CO). ORD (c=0.32, MeOH) $[\alpha]$ (nm): −47° (350), −1000° (307) (trough), 0° (293), +2513° (267) (peak), +2031° (250).

OsO_4 Oxidation of Isopropenyl Derivative (XVI) giving Glycol (XXIX)——A solution of XVI (370 mg) in anhydrous pyridine (4 ml) and anhydrous ether (40 ml) was treated with OsO_4 (290 mg) and refluxed for 4 days. During reflux, the crystals of osmate were precipitated. The crystals were collected by filtration and the filtrate was evaporated to dryness. The crystals of osmate and the residue were combined, dissolved in $CHCl_3$, and treated with stream of H_2S. After removing precipitated OsS_4, the solvent was evaporated to give a product which was then purified by neutral alumina column chromatography eluting with benzene and $CHCl_3$–AcOEt(1:1)and recrystallized with n-hexane to furnish the glycol (XXIX) (colorless needles), mp 219—221°. *Anal.* Calcd. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.96. Found: C, 72.38; H, 9.78. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 3410 (sh) (OH), 1740 (sh), 1717 ($COOCH_3$, $OCOCH_3$). NMR ($CDCl_3$) τ: 9.13 (6H, s), 9.00, 8.87, 8.83

(3H, each, all s) (totally $5\times CH_3$), 8.83 (3H, s, >C(OH) (CH_3)), 7.93, 7.90 (totlly 3H, each s),[29] *ca.* 6.6 (center, 2H, unclear signal, -CH_2OH),[29] 6.33 (3H, s, COOCH_3), *ca.* 4.8 (1H, m, >$C_{(16)}$H-OAc).

Treatment of XXIX with $Pb(OAc)_4$ affording Methylketone (XXX)——The glycol (XXIX) (100 mg) in anhydrous benzene (40 ml) was treated with $Pb(OAc)_4$ (acid free, 200 mg) with stirring at room temperature for 20 hr. After destroying excess $Pb(OAc)_4$ by adding small amount of ethyleneglycol, the reaction mixture was filtered and the filtrate was washed with water and dried. The crude product obtained after evaporation of the solvent was purified by silica gel column chromatography eluting with benzene and recrystallized with EtOH to yield methylketone (XXX) (colorless needles, 45 mg), mp 158—160°. *Anal.* Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.73; H, 9.58. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1717 (br). NMR ($CDCl_3$) τ: 9.26, 9.12, 9.00 (3H, each, s), 8.83 (6H, s) (totally $5\times CH_3$), 8.01 (3H, s, OCOCH_3), 7.90 (3H, s, COCH_3), 6.94 (1H, m, >$C_{(21)}$H-COCH_3), 6.33 (3H, s, COOCH_3), 4.58 (1H, m, >$C_{(16)}$H-OAc). ORD (c=0.24, MeOH) [M] (nm): +385° (700), +600° (589), +1242° (400), +1671° (336) (peak), +1285° (310) (trough), +7603° (250), +8138° (240) (sh), +9102° (230).

Isomerization of Methylketone (XXX) to Another Methylketone (XXXI)——A solution of XXX (20 mg) in AcOH–Ac_2O (5:1) mixture (10 ml) was refluxed in an oil bath for 4.5 hr. TLC (Silica gel G) developing with $CHCl_3$–AcOEt (1:1) revealed that XXX (Rf=0.85) was transformed into XXXI (Rf=0.91). The product obtained after the usual manner was recrystallized with *n*-hexane to afford XXXI (colorless needles), mp 185.5—187°. *Anal.* Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.41; H, 9.89. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715; ν_{max}^{KBr} cm^{-1}: 1720 (br), 1705 (sh), 1243. ORD (c=0.19, MeOH) [M] (nm): +162° (700), +487° (400), +812° (350), +2056° (302) (peak), 0° (279), −730° (265) (trough), 0° (242), +271° (231) (peak), 0° (223).

This stable methylketone (XXXI) was identified with XXXIX derived from methyl isoleucotylate (VII) later by means of mixed mp, IR ($CHCl_3$), ORD, and TLC.

Methyl 16-O-Acetyl-isoleucotylate (XXXIV)——Methyl isoleucotylate (VII) (500 mg) was treated with pyridine (22 ml) and Ac_2O (9 ml) by keeping at room temperature overnight. The product obtained after usual treatment was recrystallized with aqueous EtOH to give the acetate (XXXIV) as colorless needles (500 mg), mp 220°, $[\alpha]_D$ +70° (c=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_5$: C, 74.67; H, 10.26. Found: C, 74.54; H, 10.18. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1735 (sh), 1715. NMR ($CDCl_3$) τ: 9.18, 9.14, 9.01, 8.85 (3H, each), 8.83 (9H), (all s, totally $7\times CH_3$), 7.94 (3H, s, OCOCH_3), 6.33 (3H, s, COOCH_3), *ca.* 5.1 (1H, m, $W_{h/2}$=*ca.* 27 Hz, >$C_{(16)}$H-OAc).

Oxidation of Methyl Isoleucotylate (VII) yielding Methyl 16-Dehydro-isoleucotylate (XXXV)——Methyl isoleucotylate (VII) (630 mg) in pyridine (16 ml) was treated with CrO_3 (630 mg)–pyridine (14 ml) complex at room temperature overnight. The crude product obtained after the usual work-up was purified by alumina (20 g) column chromatography eluting with benzene. The product was then recrystallized with aqueous MeOH to give the 16-ketone derivative (XXXV) as colorless needles (500 mg), mp 212°, $[\alpha]_D$ +25° (c=0.88, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.45; H, 10.30. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1710 (COOCH_3), 1695 (CO). NMR ($CDCl_3$) τ: 9.28, 9.11, 8.96, 8.92, 8.89 (3H, each, s), 8.84 (6H, s) (totally $7\times CH_3$), 6.31 (3H, s, COOCH_3).

Dehydration of Methyl 16-O-Acetyl-isoleucotylate (XXXIV) giving Isopropenyl Derivative (XXXVII)——To a solution of the acetate (XXXIV) (500 mg) in pyridine (15 ml), was added gradually $POCl_3$ (2.5 ml) and the reaction mixture was let stand at about 5° overnight. The product obtained by usual treatment was recrystallized with EtOH to yield the isopropenyl derivative (XXXVII) as colorless crystals (420 mg), mp 221°, $[\alpha]_D$ +61.5° (c=1.3, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.29; H, 10.09. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1640, 890. NMR ($CDCl_3$) τ: 9.17, 9.14, 8.98, 8.91, 8.85 (3H, each, all s, totally $5\times CH_3$), 8.30 (3H, s, CH_3-C=CH_2), 8.14 (3H, s, OCOCH_3), 6.33 (3H, s, COOCH_3), 5.37 (2H, s, >C=CH_2), *ca.* 4.9 (1H, m, >$C_{(16)}$H-OAc).

OsO_4 Oxidation of Isopropenyl Compound (XXXVII) yielding Glycol (XXXVIII)——To a solution of XXXVII (200 mg) in anhydrous ether (30 ml)–anhydrous pyridine (3 ml) mixture, was added OsO_4 (130 mg) and the reaction mixture was refluxed for 16 hr. The residue obtained after evaporation of the solvent under reduced pressure was dissolved in $CHCl_3$ and treated with stream of H_2S. After removing precipitated OsS_4 by filtration, the product obtained from the filtrate was purified by silica gel column chromatography and recrystallized repeatedly with $CHCl_3$–*n*-hexane to give the glycol (XXXVIII) as colorless crystals (90 mg), mp 200°, $[\alpha]_D$ +72° (c=1.5, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.96. Found: C, 72.02; H, 9.78. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1740, 1712.

$Pb(OAc)_4$ Oxidation of Glycol (XXXVIII) giving Methylketone (XXXIX)——A solution of the glycol (XXXVIII) (15 mg) in anhydrous benzene (20 ml) was treated with acid free Pb $(OAc)_4$ (25 mg) with stirring at room temperature for 2 hr. After destroying excess Pb $(OAc)_4$ by adding small amount of ethyleneglycol, the reaction mixture was poured into water and extracted with ether. The product was then recrystallized with *n*-hexane to yield methylketone (XXXIX) as colorless needles (10 mg), mp 184—185°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715. NMR ($CDCl_3$) τ: 9.19, 9.13, 8.99, 8.89, 8.84 (3H, each, all s, totally $5\times CH_3$), 8.06 (3H, s, OCOCH_3),

29) These signal patterns are uncertain, probably due to the mixture of two glycols (both expressed as XXIX) possessing R and S chirality at C-22.

7.84 (3H, s, $COCH_3$), *ca.* 5.1 (1H, m, $>C_{(16)}H$-OAc). The methylketone (XXXIX) thus obtained here was identified by means of mixed mp, IR ($CHCl_3$), ORD (MeOH), and TLC (SiO_2) with the stable isomer (XXXI) formerly obtained by acid treatment of methylketone (XXX).

Mild Acid Treatment of Methyl Leucotylate (IV) giving Isopropylidene Derivative (XLIII)——A mixture of methyl leucotylate (IV) (600 mg) in conc. HCl (3 ml)–EtOH (93 ml) was refluxed in a water-bath for 5 min. The crude product obtained after the usual work-up was purified by alumina column chromatography developing with benzene and $CHCl_3$ successively. The early eluate with benzene gave methyl leucotylidienate (VI) (10 mg) and the product from the following eluate with benzene was recrystallized with *n*-hexane to yield the isopropylidene derivative (XLIII) as colorless needles (40 mg), mp 183°, $[\alpha]_D$ +58.2° ($c=0.89$, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 78.92; H, 10.52. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1710. NMR ($CDCl_3$) τ: 9.41, 9.15, 9.01, 9.00, 8.90 (3H each, all s, totally $5\times CH_3$), 8.41 (6H, s, $>C=C(CH_3)_2$), 6.45 (3H, s, $COOCH_3$), *ca.* 6.0 (1H, m, $>C_{(16)}H$-OH).

The later eluate with benzene and the eluate with $CHCl_3$ recovered the starting material (IV) (330 mg) presumably contaminated with small amount of methyl isoleucotylate (VII).

Acid Treatment of Isopropylidene Compound (XLIII) givingDiene (VI) and Methyl Isoleucotylate (VII)——Isopropylidene compound (XLIII) (200 mg) in 1% HCl–EtOH (20 ml) was refluxed in a water-bath for 4.5 hr. The product obtained after usual treatment was separated into two components by alumina (3 g) column chromatography. The benzene eluate was recrystallized with EtOH to give methyl leucotylidienate (VI, 60 mg) and further the product obtained by benzene–$CHCl_3$ (5:2) elution was recrystallized with EtOH to yield methyl isoleucotylate (VII, 58 mg).

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