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Lichen Triterpenoids. III.[1] The Final Conclusion on the Stereostructure of Zeorin and Its Correlation with Leucotylin. The Structure of Isoleucotylin

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Taking into the consideration of the established structures of two coexisting lichen triterpenoids, leucotylin(I) and zeorin(II), the re-investigation on the chemical correlation between leucotylin and ezorin has been undertaken. It has been elucidated that the authors' previous proposal[1,3] on the structures of zeorin(II*), hopane(III*), and isohopane(XXI*) should be abandoned and the final conclusion on the stereostructure of zeorin(II) in addition to its correlation with leucotylin(I) has been presented. Furthermore, the structure of isoleucotylin(XXXVI), derivable from leucotylin along with leucotylidiene(XXXV) under the acid treatment, has been elucidated.

The structure of leucotylin (I), a lichen triterpenoid isolated from *Parmelia leucotyliza* Nyl., was determined by means of the chemical investigation[3] and the X-ray analysis.[4] Based on the establishment along with the chemical correlation between leucotylin and zeorin, a coexisting triterpenoid in the same lichen, a proposal has been made[1,3] leading to the structure (II*)[5] for zeorin, which possesses an isomeric isopropanol side chain configuration at C-21 in contrast with leucotylin. Furthermore, the proposition has been extended on the geometry at C-21 of hopane presenting the structure (III*) with C-21αH configuration contrary to then believed structure III with C-21βH.[6]

As announced briefly in the previous papers,[1,3] to obtain the conclusive evidence on the stereostructure of zeorin and hopane, the X-ray analysis has been performed[7] using 6-O-*p*-bromobenzoyl-zeorin (XXII) and finally the structure of zeorin has been established as II having C-21βH configuration as same as leucotylin (I). Therefore, in view of the former chemical correlation of zeorin with hopane,[6b,c] it has become apparent that hopane should be formulated as III, which coincides not only with the prior chemical evidences[6] but with the confirmation recently made by Koyama and Nakai[8] by virtue of the X-ray analysis of adiantol B bromoacetate. Furthermore, it should be noted that the conclusion is also in good accord with the chemical findings supporting C-21βH of hopane skeleton presented by Ageta and Shiojima.[9]

1) Part II: I. Yosioka, T. Nakanishi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 291 (1969).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) I. Yosioka, T. Nakanishi, and I. Kitagawa, *Chem. Pharm. Bull.*(Tokyo), **17**, 279 (1969) (*Idem, Tetrahedron Letters*, **1968**, 1485).
4) T. Nakanishi, T. Fujiwara, and K. Tomita, *Tetrahedron Letters*, **1968**, 1491.
5) For the sake of convenience, all the formulations previously presented erroneously are numbered with asterisk(*) as revealed in the present paper. The finally established structures are numbered without asterisk.
6) *a*) G.V. Baddeley, T.G. Halsall, and E.R.H. Jones, *J. Chem. Soc.*, **1961**, 3891; *b*) I. Yosioka, T. Nakanishi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 353 (1967); *c*) Y. Tsuda, K. Isobe, S. Fukushima, H. Ageta, and K. Iwata, *Tetrahedron Letters*, **1967**, 23; *d*) Y. Tsuda and M. Hattori, *Chem. Pharm. Bull.* (Tokyo), **15**, 1073 (1967); *e*) R.E. Corbett and R.A.J. Smith, *J. Chem. Soc.* (*C*), **1967**, 1622.
7) T. Nakanishi, H. Yamauchi, T. Fujiwara, and K. Tomita, *Tetrahedron Letters*, **1971**, 1157.
8) H. Koyama and H. Nakai, *J. Chem. Soc.*(*B*), **1970**, 546.
9) H. Ageta and K. Shiojima, *Chem. Commun.*, **1968**, 1372.

These circumstances have enabled us to re-examine the experimental bases which are responsible for our previous proposal[1,3,5] and it has been elucidated that unexpected isomerization occurred during purification of the dehydration products of 6,16-di-O-acetyl-leucotylin (XXXI) had brought out the erroneous results described in our previous papers.[1,3] The present paper deals with the detailed account on the subjects.[10] In addition, it offers the chemical evidence corroborating the structure (XXXVI) for isoleucotylin, which has been derived from leucotylin (I) along with leucotylidiene (XXXV) under the acid treatment.

The experimental bases,[1,3] including the correlation of zeorin with leucotylin which had led us to assume the uncorrect conclusion (II*) for zeorin, are summarized in Chart 1. The key intermediate connecting both triterpenoids was the monoketone (XI*=XX*) derivable from leucotylin and zeorin. Especially of importance was that XI* was believed to retain the C-21 geometry of leucotylin (I), which consequently resulted in the presumption of the C-21αH configuration of zeorin (II*). Namely, the monoketone (XI*) holding the C-21 geometry of leucotylin (I) was found unidentical with the monoketone (XIX*) possessing the same C-21 configuration as zeorin, but it was identified with the other monoketone (XX*) carrying the reversed configuration at C-21.

Nevertheless, since zeorin has been elucidated as II possessing the C-21βH configuration by the X-ray study,[7] there must have been serious inconsistency during the previous derivations either from leucotylin (I) to XI* or from zeorin to XX*. Considering again all the reaction sequences given in Chart 1, the essential inconsistency has been presumed to lie most likely in the assignment of IV*, since the derived norketone (VIII*) was unaffected by the acid treatment contrary to the anticipation.[6a,11,12] The assumption has now been verified as described below.

Dehydration of 6-O-acetyl-zeorin (XXIII) using $POCl_3$–pyridine followed by preparative thin-layer chromatographic (TLC) separation (silica gel impregnated with silver nitrate) furnished an isopropenyl compound (XXIV), $C_{32}H_{52}O_2$, mp 199—199.5°, and an isopropylidene derivative (XXV), $C_{32}H_{52}O_2$, mp 123—123.5°. The nuclear magnetic resonance (NMR) spectra of both substantiate the respective formulations by the signals at 8.22 τ (3H, s) and 5.17 τ (2H, *t*-like) for the isopropenyl function of XXIV and at 8.42 τ (6H, s) for the isopropylidene function of XXV. Ozone oxidation of XXIV gave an unstable norketone (XXVI), $C_{31}H_{50}O_3$, mp 219—221°, whose constitution is supported by its infrared (IR) absorption bands (CCl_4, cm^{-1}) at 1734, 1246 (OAc), and 1712 (CO) and the NMR singals at 7.98 and 7.90 τ (3H each, s, $OCOC\underline{H}_3$ and $COC\underline{H}_3$). The norketone (XXVI) was readily isomerized by refluxing in AcOH–Ac_2O mixture to another stable norketone (XXVII), $C_{31}H_{50}O_3$, mp 224—226.5°. The remarkable difference of both norketones is observed in the circular dichroism (CD) curves. The former (XXVI) exhibits a strong positive maximum (θ: +4210 at 292 nm) while the latter (XXVII) a weak positive maximum (θ: +840 at 292 nm) in dioxane solution. These observations are well explained by the increased sterical congestion anticipated between the methylketone and C-18α methyl groups in the former unstable norketone (XXVI), as were interpreted the CD data of adiantone (unstable, θ: +3620) and isoadiantone (stable, θ: +260).[13] Accordingly, the isopropenyl derivative (XXIV) and the derived norketone (XXVI) are believed to hold the C-21βH configuration of zeorin (II).

Moreover, hydrogenation of XXIV in the neutral solvent afforded a saturated compound (XXVIII), $C_{32}H_{54}O_2$, mp 181.5—183°, which in turn was transformed to a monoketone (XXX) by alkaline hydrolysis followed by the Kiliani oxidation (*via* an alcohol

10) I. Yosioka, T. Nakanishi, H. Yamauchi, and I. Kitagawa, *Tetrahedron Letters*, **1971**, 1161 (Preliminary account on the subjects.).
11) G. Berti, F. Bottari, A. Marsili, J.M. Lehn, R. Witz, and G. Ourisson, *Tetrahedron Letters*, **1963**, 1283.
12) I. Yosioka, M. Yamaki, T. Nakanishi, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 2227.
13) P. Crabbé, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Holden-Day, San Francisco, 1965, p. 145.

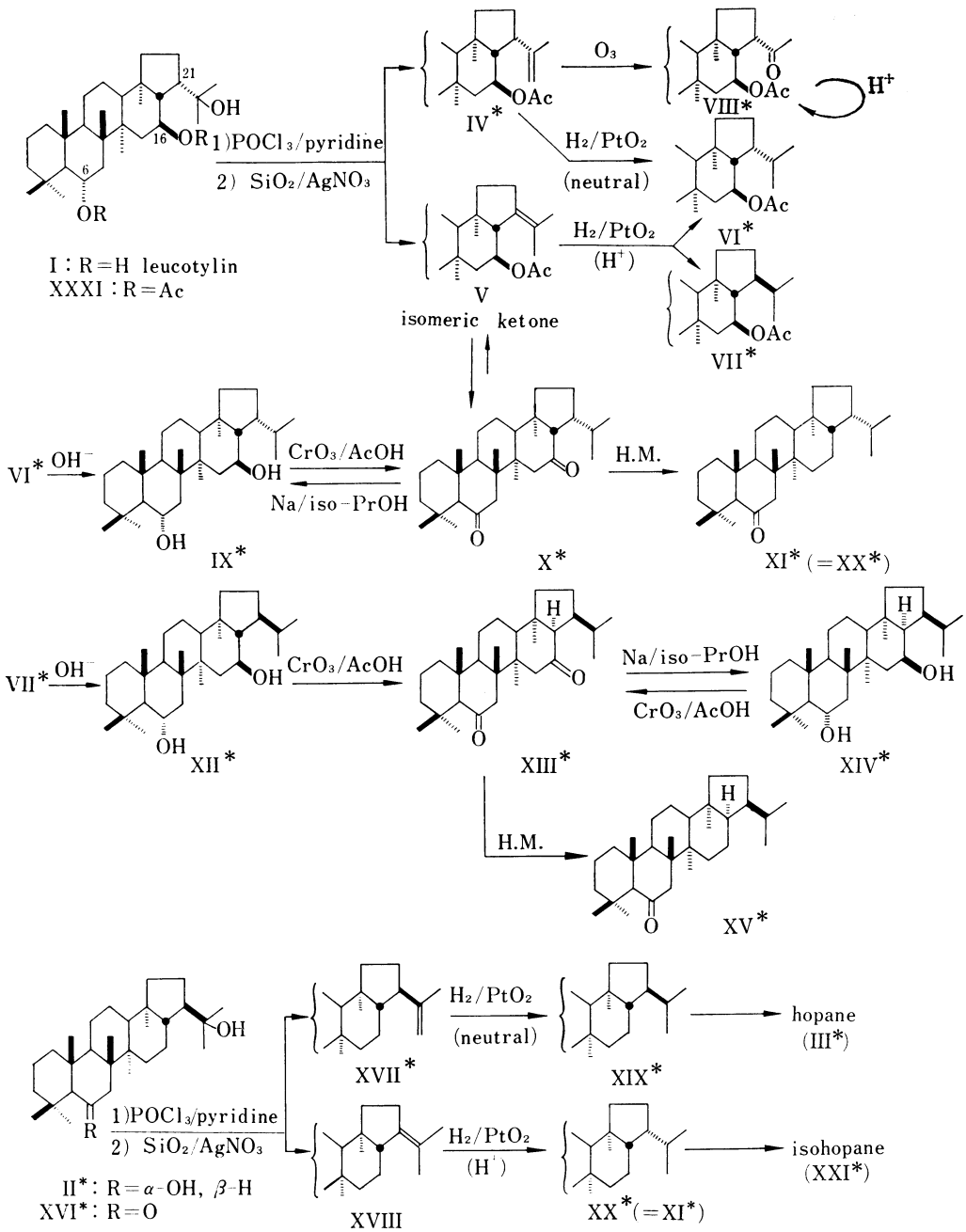
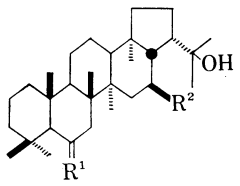


Chart 1. Previous Assignment[1,3,5]

(XXIX)). The monoketone (XXX) thus obtained was found identical with the previously reported monoketone (XIX) (former assignment XIX* in Chart 1)[1] by mixed mp, IR, and TLC. Therefore, all the derivatives of zeorin including the previous experiments are now correctly formulated as given in Chart 2.[14]

14) The newly performed derivations as described in the present paper are led with the thick arrows.

In other words, it follows that the former assignment of C-21βH of the key intermediate (XX* from zeorin and XI* from leucotylin in Chart 1) should be inverted to C-21αH (as in XX=XI) as illustrated in Chart 2. The present revised conclusion is in agreement with the fact[6b,c] that XX has been transformed to isohopane (XXI) whereas the isomeric monoketone (XIX) to hopane (III) whose constitution was established by the recent X-ray analysis.[8]



I : $R^1=\alpha$-OH, β-H; R^2=OH leucotylin
II : $R^1=\alpha$-OH, β-H; R^2=H zeorin
XVI : R^1=O; R^2=H zeorinone
XXII : $R^1=\alpha$-O-*p*-bromobenzoyl, β-H; R^2=H
XXIII : $R^1=\alpha$-OAc, β-H; R^2=H
XXXI : $R^1=\alpha$-OAc, β-H; R^2=OAc

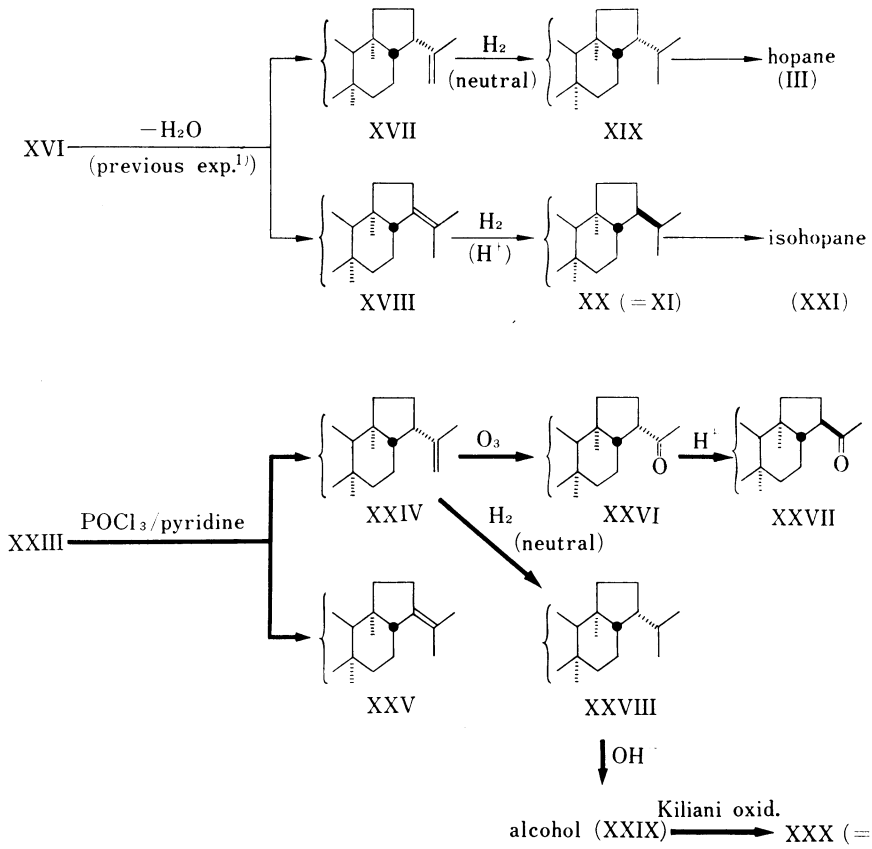


Chart 2. Established Formulations of Zeorin Derivatives. The Derivations led with Thick Arrows indicate the Present Experiments

Since the stereostructure of XX (=XI) has been established, it has been suspected that unexpected inversion of the C-21 geometry must have taken place during the previous procedure from 6,16-di-O-acetyl-leucotylin (XXXI, with C-21βH) to XI (with C-21αH). The re-investigation along this line has been undertaken.

In the previous experiment,[3] dehydration of XXXI with $POCl_3$–pyridine followed by column chromatographic separation using silica gel impregnated with silver nitrate yielded

the isopropenyl (IV*) and the isopropylidene (V) derivatives (Chart 1). In the present examination, however, the treatment of XXXI under the same reaction condition followed by preparative TLC (silica gel impregnated with silver nitrate) separation furnished, in addition to the known isopropylidene derivative (V), a new isopropenyl compound (XXXII), $C_{34}H_{54}O_4$, mp 219—220°, which is completely distinguishable from the former isopropenyl derivative IV* (now formulated IV as mentioned below), $C_{34}H_{54}O_4$, mp 210—211°.[15] The noticeable differences among the physical data of both are that the C=C stretching absorption band is observed stronger at higher wave number (1642 cm^{-1}) in IV than in XXXII (1630 (br) cm^{-1}) while the NMR signal due to the endomethylene of the isopropenyl function is observed at higher field (5.36 τ) in IV than in XXXII (5.29 τ).

Ozone oxidation of the new isopropenyl derivative (XXXII) yielded a new unstable norketone (XXXIII), $C_{33}H_{52}O_5$, mp 229—230°, CD (MeOH): $[\theta]_{288}$—2739 (negative maximum), which was isomerized readily under reflux in AcOH–Ac_2O mixture to another stable norketone (XXXIV), mp 218—221°, CD (MeOH): $[\theta]_{283}$+2010 (positive maximum).[16] It is noteworthy to point out here that the latter norketone (XXXIV) was found identical (mixed mp, IR (KBr), optical rotatory dispersion (ORD), and TLC) with the norketone (VIII, previous assignment VIII*), which was previously prepared *via* ozone oxidation from the former isopropenyl derivative (now assigned IV) and was noticed stable against the acid treatment. In view of these evidences and referring to the chemical properties of the norketones (XXVI, XXVII, adiantone,[11] and isoadiantone[11]), the new norketone is analogously assigned as XXXIII carrying C-21βH configuration (as XXVI and adiantone) and the stable norketone as XXXIV (=VIII) (as XXVII and isoadiatone).[17] Consequently, it is concluded that XXXII possesses the C-21βH geometry as XXXI.

Furthermore, hydrogenation of XXXII over the Adams' catalyst under the neutral condition afforded the diacetate (VII), which is isomeric to another saturated compound (IV) derivable from the formerly obtained isopropenyl derivative (IV) under the same reaction condition. This clearly discloses that the previous isopropenyl compound prepared from 6,16-di-O-acetyl-leucotylin (XXXI) possesses the inverted configuration at C-21 (as IV) rather than IV* (with C-21βH).

Accordingly, the chemical correlation between leucotylin and zeorin has now been established in all respects as illustrated in Chart 2 and 3. All the formulae with asterisk (*) (in Chart 1) must be revised to the formulae of the same numbering without asterisk. We have to withdraw our earlier claim for the structures II*, III*, and XXI* for zeorin, hopane, and isohopane. Concerning the fact that the isopropenyl derivative (IV) was no more preserving the original C-21 configuration of leucotylin (I), it has been presumed that unexpected isomerization had taken place during the prolonged separation procedure using SiO_2-$AgNO_3$ column in our previous experiment.[3] Although we have been unable to repeat the previous results exactly, the re-examination on this point has suggested that XXXII is possibly isomerized if partly to IV during the chromatographic procedure.

In addition, the chemical behavior of the carbonyl derivatives (now formulated X and XIII) is better explained by the present assignments than by the previous ones.[3] Thus, the diketone (X) (having D/E *trans* and C-21αH) is quite stable against either the acid or

15) For the physical data, see the experimental section. Further TLC survey of the reaction mixtures (mother liquors *etc.*) of the former experiment[3] disclosed the existence of a tiny amount of XXXII.

16) Although the true reason is obscure, the different trend between the molecular ellipticity of leucotylin derivatives(XXXIII, XXXIV) and that of zeorin derivatives(XXVI, XXVII) might be ascribed to the existence of bulky C-16β-OAc in the formers, since the group is presumed to affect increasingly the conformation of the methylketone moiety.

17) Previous considerations (lit. 3, footnote 39.) concerning the relative stability of the methylketone moiety attached to C-21 should now be replaced by the simple explanation ascribing to the sterical interaction between C-18α methyl and C-21α methylketone expected in XXVI, XXXIII, or adiantone.[11]

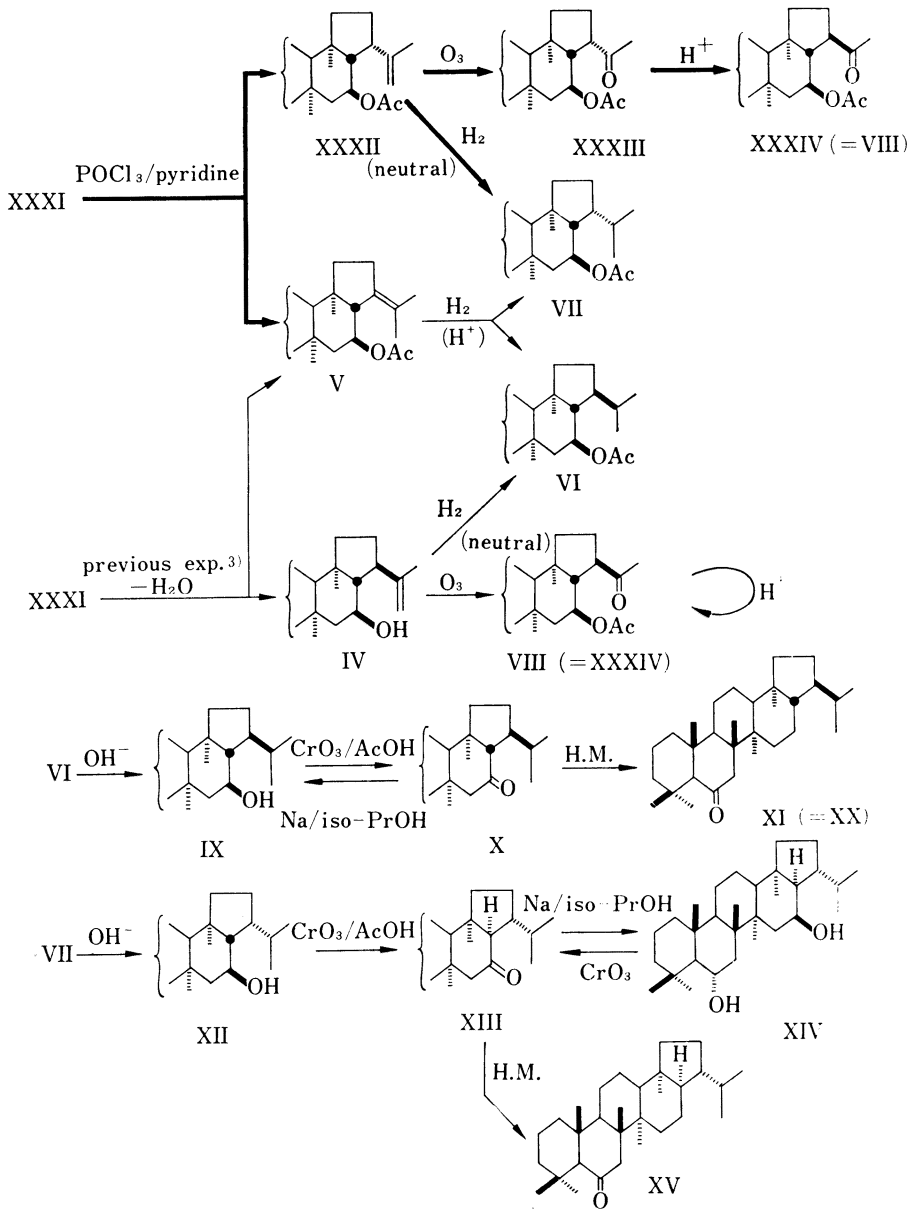


Chart 3. Established Formulations of Leucotylin Derivatives. The Derivations led with Thick Arrows indicate the Present Experiments

alkali treatment, since the formation of the D/E *cis* isomer is prevented by the severe sterical interaction anticipated between the bulky C-21β isopropyl side chain and C-15 methylene as was discussed in case of leucotylic acid derivatives.[12,18] On the other hand oxidation of the diol (XII) (with D/E *trans* and C-21βH) under the acidic condition furnished only the isomerized diketone (with D/E *cis* and C-21βH) in poor yield, which implies that the awaited diketone (XIII, but with D/E *trans*) is unstable due to well known instability of the

18) D.H.R. Barton and G.A. Morrison, "Fortschritte der Chemie Organischer Naturstoffe," Vol. 19, ed. by L. Zechmeister, Springer-Verlag, Vienna, 1961,

trans indanone framework. Contrary to X, the *cis* indanone structure (XIII) is not obstracted by the C-21α isopropyl side chain.

Eventually, taking into the consideration of the final results visualized in Charts 2 and 3, the following designations presented in the previous papers[1,3] should be replaced by the names based on the correct formulations: 6,16-di-O-acetyl-22-desoxy-leucotylin→6,16-di-O-acetyl-22-desoxy-21αH-leucotylin (VI); 6,16-di-O-acetyl-22-desoxy-21αH-leucotylin→6,16-di-O-acetyl-22-desoxy-leucotylin (VII); 22-desoxy-leucotylin→22-desoxy-21αH-leucotylin (IX); 6,16-diketo-22-desoxy-leucotylin→6,16-diketo-22-desoxy-21αH-leucotylin (X); 16,22-dideso-xy-6-dehydroleucotylin (=22-desoxy-21βH-zeorinone)→16,22-didesoxy-21αH-6-dehydroleucotylin (XI) (=22-desoxy-21αH-zeorinone (XX)); 22-desoxy-21αH-leucotylin→22-desoxy-leucotylin (XII); 6,16-diketo-22-desoxy-17αH,21αH-leucotylin→6,16-diketo-22-desoxy-17αH-leucotylin (XIII); 22-desoxy-17αH,21αH-leucotylin→22-desoxy-17αH-leucotylin (XIV).

It was reported in the previous paper[3] that reflux of leucotylin (I) in 5% ethanolic hydrogen chloride furnished leucotylidiene (XXXV). Further survey of the reaction product has led to isolate, in addition to the diene, an isomeric compound now named isoleucotylin (XXXVI), $C_{30}H_{52}O_3$, mp 266—268°, was was experienced in the acid treatment of methyl leucotylate (XXXVIII), where methyl isoleucotylate (XXXIX) was obtained along with the diene (XL).[12] The structure of isoleucotylin has been elucidated as below (Chart 4).

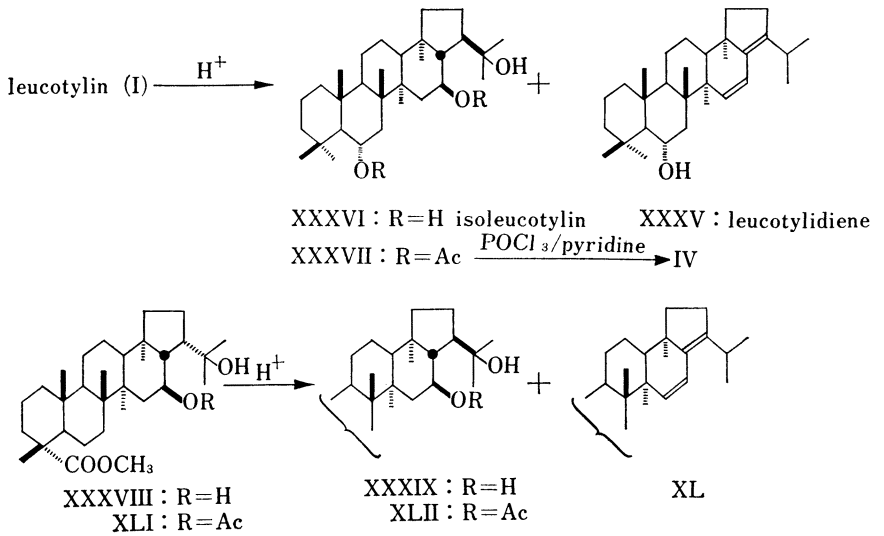


Chart 4

Although the isomer moves similarly as leucotylin on TLC, they are distinguishable by detection with 1% $Ce(SO_4)_2$/10% H_2SO_4 under heating. Acetylation of XXXVI smoothly yielded a diacetate (XXXVII), $C_{34}H_{56}O_5$, mp 236—237.5°, which is discriminated from 6,16-di-O-acetyl-leucotylin (XXXI), mp 241—242°, by mp, IR, NMR, and TLC comparisons.

The most significant difference of both acetates in the NMR data is that a methyl signal appearing at the highest field (probably due to C-18α methyl) of XXXVII is observed at 9.21 τ while in XXXI at 9.15 τ. The similar tendency is also experienced in case of methyl 16-O-acetyl-isoleucotylate (XLII) (9.18 τ)[12] and methyl 16-O-acetyl-leucotylate (XLI) (9.14 τ).[19]

Finally, dehydration of XXXVII with $POCl_3$–pyridine afforded an isopropenyl compound as a sole product, which was found identical with IV by mixed mp, IR (CCl_4), and

19) I. Yosioka, T. Nakaninishi, and E. Tsuda, *Tetrahedron Letters*, **1966**, 607.

TLC, thus confirming the structure of isoleucotylin to be XXXVI. The formation mechanism of XXXVI is analogously assumed as discussed for methyl isoleucotylate (XXXIX).[12]

Experimental[20]

Zeorin Derivatives

6-O-*p*-Bromobenzoyl-zeorin(XXII)——To a solution of zeorin(II) (406 mg) in pyridine (10 ml), was added gradually *p*-bromobenzoyl chloride (2 ml) under ice cooling. The solution mixture was let stand at 30° for 18 hr. Crude benzoate obtained after treating in a usual manner was then purified by neutral alumina (Woelm grade III) column chromatography (developing with benzene) followed by preparative TLC(SiO_2, $CHCl_3$) purification and recrystallization with benzene–acetone mixture affording *p*-bromobenzoate (XXII) (colorless needles, 200 mg), mp 224—227°. *Anal.* Calcd. for $C_{37}H_{55}O_3Br$: C, 70.79; H, 8.83; Br, 12.73. Found: C, 70.61; H, 8.96; Br, 12.91. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3607, 1715, 1588, 1247. NMR($CDCl_3$)τ: 9.25 (3H, s), 9.16 (3H, s), 9.03 (6H, s), 8.98 (3H, s), 8.87 (6H, s), 8.83 (3H, s) (totally $8\times CH_3$), 4.52 (1H, m, $>C_{(6)}H$-O-*p*-bromobenzoyl), 2.50, 2.16 (4H, A_2B_2, phenyl ring protons).

Alkaline Hydrolysis of *p*-Bromobenzoate——The *p*-bromobenzoate (XXII) (42 mg) was treated with 5% KOH–MeOH(35 ml) under reflux in a water-bath for 2 hr. The hydrolysate obtained after the usual work-up and preparative TLC yielded pure zeorin(II) (21 mg), identical with the authentic sample by mixed mp, IR(KBr) and TLC.

Dehydration of 6-O-Acetyl-zeorin(XXIII) with $POCl_3$ yielding Isopropenyl(XXIV) and Isopropylidene (XXV) Derivatives——To an ice-cooled solution of 6-O-acetyl-zeorin(XXIII) (1.5 g) in pyridine (30 ml) was added gradually $POCl_3$–pyridine solution (5.7 ml–5.7 ml) with stirring and the total solution was kept at about 5° for 18 hr. The reaction mixture (1.4 g) obtained after the usual way consisted of two major components namely isopropenyl and isopropylidene derivatives in addition to a trace amount of a minor one as revealed by TLC. A part(0.2 g) of this mixture was separated by preparative TLC with silver nitrate impregnated silica gel developing with benzene and the each fraction was eluted from the adsorbent using benzene. The least polar fraction was recrystallized with MeOH giving an isopropylidene derivative(XXV), mp 123—123.5°. *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.70; H, 11.34. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1738, 1520 (OAc). NMR ($CDCl_3$) τ: 9.40, 9.13, 9.05, 9.00, 8.95, 8.88 (3H each all, s, $6\times CH_3$), 8.42 (6H, s, $>C=C(CH_3)_2$), 7.98 (3H, s, $OCOCH_3$), 4.75 (1H, d of t, $J=11.5$ & 7.5Hz, $>C_{(6)}H$–OAc). On recrystallization with MeOH, the second less polar component yielded a pure isopropenyl derivative(XXIV), mp 199—199.5.° *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.84; H, 11.14. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1253 (OAc), 1643, 890 ($>C=CH_2$). NMR($CDCl_3$) τ: 9.23, 9.10, 9.03, 9.00, 8.96, 8.85 (3H each, s, $6\times CH_3$), 8.22 (3H, s, $C=C-CH_3$), 7.95 (3H, s, $OCOCH_3$), 5.17 (2H, *t*-like, $>C=CH_2$), 4.71(1H, d of t, $J=11.5$ & 7.5 Hz, $>C_{(6)}H$–OAc).

Ozone Oxidation of Isopropenyl Derivative(XXIV) giving Norketone(XXVI)——An ozonide mixture, prepared from the isopropenyl derivative(XXIV)(50 mg) in *n*-hexane–EtOH(2: 1) solution(10 ml) with slow stream of ozone for 2 hr under ice-cooling, was diluted with additional 10 ml of n-hexane–EtOH(1:1) mixture and was hydrogenated over PtO_2(50 mg) for 4 hr. After removing the catalyst by filtration, the product obtained by evaporation of the solvent was crystallized from aqueous MeOH to give a norketone(XXVI) (35 mg), mp 219—221°. *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.00; H, 10.68. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1734, 1246(OAc), 1712(CO). NMR($CDCl_3$) τ: 9.40, 9.14, 9.07, 9.05, 8.97, 8.89(3H each, s)(totally $6\times CH_3$), 7.98(3H, s, $OCOCH_3$), 7.90(3H, s, $COCH_3$), *ca.* 4.8(1H, m, $>C_{(6)}H$–OAc). CD ($c=0.24$, dioxane) $[\theta]^{24}$(nm): +4210 (292) (positive maximum).

Isomerization of Norketone(XXVI) giving Isonorketone(XXVII)——A solution of norketone(XXVI) (16 mg) in AcOH(3 ml)–Ac_2O(0.6 ml) mixture was refluxed for 4.5 hr in an oil bath. The product was then purified by preparative TLC(SiO_2, developing with benzene) and recrystallized with aqueous EtOH to give isonorketone(XXVII) (colorless needles), mp 224—226.5°. *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 78.78; H, 10.79. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1246 (OAc), 1714 (CO). NMR($CDCl_3$) τ: 9.30, 9.14, 9.06, 9.03, 8.97, 8.89(3H each, s)(totally $6\times CH_3$), 7.99 (3H, s, $OCOCH_3$), 7.88 (3H, s, $COCH_3$), 4.77(1H, d of t, $J=11.0$ & 7.5 Hz, $>C_{(6)}H$–OAc). CD($c=0.24$, dioxane) $[\theta]^{24}$ (nm): +840(292) (positive maximum).

Hydrogenation of Isopropneyl Compound(XXIV) giving 6-O-Acetyl-22-desoxy-zeorin(XXVIII)——A solution of isopropenyl derivative(XXIV) (36 mg) in *n*-hexane (10 ml) and EtOH (20 ml) mixture was hydrogenated over PtO_2 (35 mg) at room temperature for 1.5 hr. The product, after recrystallization with $CHCl_3$–MeOH, yielded XXVIII(colorless needles, 32 mg), mp 181.5—183.5°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1245(OAc). *Anal.* Calcd. for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81.84; H, 11.78. NMR ($CDCl_3$)τ: 9.29, 9.15, 9.07, 9.05,

20) Melting points were taken on the Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and recorded uncorrected. NMR spectra were recorded on a Varian A-60 Spectrometer using tetramethylsilane as an internal standard and IR spectra on a Hitachi EPI-G31, EPI-G21 or EPI-S2 Spectromter. CD and ORD data were obtained on a JASCO ORD/UV-5 Automatic Recording Spectropolarimeter.

8.97, 8.92 (3H each, s,) 9.10 (6H, d, $J=10$ Hz) (totally $8\times CH_3$), 8.00(3H, s, $OCOCH_3$), 4.76(1H, d of t, $J=$ 11.2 & 7.3 Hz, $>C_{(6)}H$–OAc).

Alkaline Hydrolysis of XXVIII giving 22-Desoxy-zeorin(XXIX)——XXVIII (22 mg) was treated with KOH–MeOH–dioxane(0.6 g–4 ml–3.5 ml) mixutre under reflux for 9.5 hr. The crude product which contained a small amount of the starting material (XXVIII) was purified by preparative TLC(SiO_2) and recrystallized with aqueous EtOH to give XXIX (colorless needles, 7 mg), mp 194—196°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3465 (br) (OH). NMR ($CDCl_3$) τ: 9.29, 9.13 (3H each, s), 9.05 (6H, d, $J=9$ Hz), 9.03 (6H, s), 8.97 (6H, s) (totally $8\times CH_3$), 6.03 (1H, m, $>C_{(6)}H$–OH).

Oxidation of XXIX giving 22-Desoxy-zeorinone (XXX=XIX)——To an ice-cooled solution of XXIX (4 mg) in acetone, was added gradually the Kiliani mixture (4 drops) and the total mixture was stirred for 15 min under ice-cooling conditiom. The crude product, after preparative TLC(SiO_2) purification followed by recrystallization with aqueous EtOH, yielded XXX (3 mg). XXX thus obtained was identified with XIX prepared previously from zeorinone(XVI)[1] by means of mixed mp, IR (KBr), and TLC.

Leucotylin Derivatives

Dehydration of 6,16-Di-O-acetyl-leucotylin(XXXI) with $POCl_3$ yielding New Isopropenyl(XXXII) and Isopropylidene (V) Derivatives——To a solution of diacetate (XXXI) (2 g) in pyridine (50 ml), was added gradually $POCl_3$ (8 ml) under ice-cooling and the reaction mixture was let stand at *ca.* 5° overnight. TLC (using SiO_2–$AgNO_3$) of the reaction mixture (1.8 g) obtained after the usual work-up revealed to consist of a new isopropenyl(XXXII) and the isopropylidene (V) compounds. A part (200 mg) of the mixture was then separated quickly by preparative TLC(SiO_2–$AgNO_3$) developing with $CHCl_3$–AcOEt (100:1) to isolate two components. The less polar component on recrystallization with MeOH yielded the isopropylidene derivative (colorless needles, 80 mg), mp 181.5—182.5°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1741 (sh), 1737, 1247 (OAc), which was identified with the isopropylidene derivative (V) otained before[3] by mixed mp, IR (CCl_4), and TLC(SiO_2–$AgNO_3$). On recrystallization with MeOH, another component gave a new isopropenyl derivative (XXXII) (colorless needles, 60 mg), mp 219—220.° *Anal.* Calcd. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.12; H, 10.77. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1741 (sh), 1737, 1247 (OAc), 1630 (br), 890 ($>C=CH_2$). NMR($CDCl_3$) τ: 9.14 (6H, s), 9.06 (3H, s), 8.96 (3H, s), 8.90 (6H, s) (totally $6\times CH_3$), 8.30 (3H, s, C=C–CH_3), 8.06, 7.99 (3H each, s, $2\times OCOCH_3$), 7.12 (1H, m, $>C_{21}$–H–), 5.29 (1H, br s, $>C=CH_2$), ca. 4.8 (2H, m, $2\times>CH$–OAc). XXXII is obviously distinguishable from the previous isopropenyl derivative (now formulated IV)[3] by means of mp, IR (CCl_4), NMR, and TLC (SiO_2–$AgNO_3$). The physical data of IV[3]: mp 210—211°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1741 (sh), 1737, 1257 (OAc), 1642, 887 ($>C=CH_2$). NMR ($CDCl_3$) τ: 9.18, 9.14, 9.04, 8.96, 8.92, 8.83 (3H each, s, $6\times CH_3$), 8.28 (3H, s, C=C–CH_3), 8.12, 7.97, (3H each, s, $2\times OCOCH_3$), 5.36 (2H, s, $>C=CH_2$), *ca.* 4.9 (2H, m, $2\times>CH$–OAc).

Ozone Oxidation of Isopropenyl Derivative(XXXII) giving Norketone(XXXIII)——An ozonide mixture was prepared from the isopropenyl compound(XXXII) (37 mg) in *n*-hexane (12 ml) with slow stream of ozone for 3.5 hr under the ice cooling condition. After diluting with EtOH (15 ml) and *n*-hexane (10 ml), the ozonide mixture was hydrogenated over PtO_2 (100 mg) for 2.2 hr, and the catalyst was removed by filtration. The product obtained by evaporation of the solvent was purified by preparative TLC(SiO_2) and then crystallized from aqueous MeOH to give a norketone (XXXIII) (25 mg), mp 229—230°. *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 74.92; H, 10.10. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1725(sh), 1246 (OAc, CO). CD($c=0.21$, MeOH)$[\theta]^{23}$(nm): -2739(288) (negative maximum), ORD($c=0.21$, MeOH)[M]^{23}(nm): 0.25×10^3(700), 0.88×10^3(589), 2.39×10^3(400), 3.52×10^3(331) (broad peak alike), 3.27×10^3(317) (trough), 11.57×10^3(250) (peak), 11.69×10^3(240). The norketone was distinguishable by mp, IR (KBr), and ORD) from the previous norketone (VIII)[3] prepared from the isopropenyl compound (IV) by ozone oxidation.

Isomerization of Norketone(XXXIII) giving XXXIV Identical with Previous Norketone(VIII)——A mixture of the norketone(XXXIII) (4 mg) in AcOH–Ac_2O (5:1) (3 ml) was refluxed in an oil bath (bath temp. 135—145°) for 4.5 hr. The product, on recrystallization with aqueous MeOH, gave an isomer(XXXIV) (colorless needles), mp 218—221°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1744 (sh), 1740, 1725 (sh), 1248 (OAc, CO). CD ($c=$ 0.26, MeOH) $[\theta]^{23}$(nm): $+2010$ (283) (positive maximum). The isomer(XXXIV) was identified with the former norketone(VIII)[3] prepared from the previcus isopropenyl compound(IV) through ozone oxidation by means of mixed mp, IR (KBr), ORD (MeOH), and TLC comparisons. The reported ORD data[3] of the former norketone(VIII) (in lit. 3: compound XX) was uncorrect due to the careless mistake. The authors would like to reform them with the sincere apology as follows: ORD ($c=0.26$, MeOH) [M]^{23}(nm): 0.51×10^3(700), 0.61×10^3(589), 3.76×10^3(298) (peak), 2.34×10^3(270) (trough), 2.64×10^3(246) (peak), 2.44×10^3 (233), 6.60×10^3(220).

Hydrogenation of Isopropenyl Compound(XXXII) in Neutral Medium giving 6,16-Di-O-acetyl-22-desoxy-leucotylin(VII)——The isopropenyl derivative(XXXII) (20 mg) in EtOH (35 ml) was hydrogenated over PtO_2 (30 mg) at room temperature for 2.2 hr. On recrystallization with MeOH, the product yielded 6,16-di-O-acetyl-22-desoxy-leucotylin(VII) (colorless needles, 18 mg), mp 231—232°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1742 (sh), 1738, 1248 (OAc), which was not identical with the saturated derivative(VI) obtained from the former isopropenyl compound(IV) by neutral medium hydrogenation,[3] but identical with the other saturated compound (VII) obtained from the isopropylidene derivative (V) by acid medium hydrogenatiion,[3] by means of mixed mp, IR (CCl_4), and TLC comparisons.

The Attempts to clarify the Ambiguity in the Previous Experiment——(1) On the Quality of $POCl_3$: Three kinds of $POCl_3$ ((i) special grade, freshly re-distilled before use, bp 108°, (ii) practical grade, re-distilled, (iii) practical grade, without re-distillation) were used for the dehydration reaction, however, the results did not show any significant difference as revealed by the product analysis. The former isopropenyl compound (IV) was not detected in any case.

(2) Temperature Dependence: Since the previous dehydration was run at room temperature,[3] the reaction was examined at room temp. (31°), at 35°, or at 5° by keeping overnight. However, the each product was disclosed to consist of XXXII and V, and not to contain IV.

(3) Possible Isomerization during SiO_2–$AgNO_3$ Column Chromatography: The dehydrated mixture (200 mg), whose composition was disclosed to be XXXII and IV by TLC, was adsorbed on SiO_2 (Mallinckrodt, same as before[3])–$AgNO_3$[21] (20 g) with the aid of *n*-hexane–benzene(9:1) mixture and eluted with *n*-hexane–benzene mixture(9:1→1:1) and benzene successively during the period of 3 weeks (previous exp. *ca.* 4 weeks). The combined eluates of *n*-hexane–benzene(3:1) and earlier (1:1) gave V (70 mg). The following *n*-hexane–benzene(1:1) mixture eluted out a mixture of V and XXXII, while the later eluate of 1:1 mixture and earlier eluate of benzene furnished XXXII (45 mg). The successive benzene elution gave a mixture of XXXII and IV, from which pure IV(3 mg) was isolated by preparative TLC(SiO_2–$AgNO_3$). It is till suspected that $AgNO_3$ previously used might also be responsible, however, we were unable to repeat exactly the previous experiment.[3]

Acid Treatment of Leucotylin(I) giving Isoleucotylin(XXXVI) together with Leucotylidiene(XXXV) Leucotylin (I) (200 mg) was refluxed in conc. HCl (5.5 ml)–EtOH (28 ml) mixture for 20 min. The reaction mixture was diluted with water and extracted with ether. The residue obtained by evaporation of the solvent consisted of two components (revealed by TLC) and was chromatographed on silica gel column. The eluate with $CHCl_3$–benzene (2:1) mixture was recrystallized with aqueous EtOH to afford the known diene (XXXV) (120 mg), mp 167.5—168.5.[3] Further elution with MeOH followed by recrystallization with MeOH yielded isoleucotylin (XXXVI) (colorless feathery crystals, 60 mg), mp 266—268°. *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found: C, 78.51; H, 11.25. IR ν_{max}^{KBr} cm^{-1}: 3226, 3257 (sh) (OH) (*cf.* leucotylin(I)[3]: IR ν_{max}^{KBr} cm^{-1}: 3226). Isoleucotylin (XXXVI) and leucotylin(I) move with the same *Rf* value on TLC(SiO_2), however the former colored dark brown with 1% $Ce(SO_4)_2$/10% H_2SO_4 under heating, while the latter purple.

6,16-Di-O-acetyl-isoleucotylin(XXXVII)——Isoleucotylin(XXXVI) (20 mg) was treated with pyridine (6.5 ml) and Ac_2O (2 ml) at room temperature overnight. The product was recrystallized with *n*-hexane–MeOH to give the diacetate(XXXVII) (colorless needles, 20 mg), mp 236—237.5°. *Anal.* Calcd. for $C_{34}H_{56}O_5$: C, 74.95; H, 10.36. Found: C, 75.27; H, 10.10. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3530 (OH), 1750 (sh), 1730, 1242 (OAc). NMR ($CDCl_3$) τ: 9.21, 9.16, 9.08, 8.97 (3H each, s), 8.88, 8.84 (6H each, s) (totally $8\times CH_3$), 7.98, 7.95 (3H each, s, $2\times OCOCH_3$), *ca.* 5.0 (2H, m, $2\times >CH$-OAc), which was distinguishable from 6,16-di-O-acetyl-leucotylin (XXXI) by IR (CCl_4), NMR, and TLC (SiO_2). For example, 6,16-di-O-acetyl-leucotylin (XXXI)[3]: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3550, 1750 (sh), 1733, 1242.

Dehydration of 6,16-Di-O-acetyl-isoleucotylin (XXXVII) with $POCl_3$ yielding Isopropenyl Derivative (VI) To an ice-cooled solution of the diacetate (XXXVII) (12 mg) in pyridine (6 ml), was pured slowly $POCl_3$ (1 ml) and the total solution was let stand at *ca.* 5° overnight and treated in a usual manner. The product (single component as revealed by TLC) was recrystallized with MeOH to yield an isopropenyl derivative (colorless needles), mp 212—213°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1741 (sh), 1737, 1247, 1642, 887, which was identified with the isopropenyl derivative (IV) formerly obtained from XXXI[3] by mixed mp, IR (CCl_4), and TLC (SiO_2–$AgNO_3$).

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21) T. Norin and L. Westfelt, *Acta Chem. Scand.*, **17**, 1828 (1963).