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Lichen Triterpenoids. V.[1] On the Neutral Triterpenoids of *Pyxine endochrysina* Nyl.

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As a continuation of the study on the lichen triterpenoids, the neutral triterpenoids of a lichen *Pyxine endochrysina* Nyl. have been investigated. The structure of diacetylpyxinol, a major component, has been established as 3β, 25-diacetoxy-12β-hydroxy-20(*S*),-24(*R*)-epoxy-dammarane (II) on the basis of the chemical evidence and the X-ray analysis. In addition, the other minor neutral components have also been elucidated as pyxinol (III), 12-desoxy-diacetylpyxinol (IV), 3-O-acetyl-pyxinol (V), methyl pyxinate (VI), and methyl 3-O-acetyl-pyxinate (VII), among which the latter two compounds possess the hopane skeleton.

Diacetylpyxinol and the analogues are the first examples of the dammarane triterpenoids discovered in the lichen family.

In recent years, it has become acquainted that triterpenoids distribute fairly widely in the lichen family.[3] As a continuation of the investigation on the lichen triterpenoids, we have previously studied on the constituents of a lichen *Pyxine endochrysina* Nyl. and elucidated the structure of pyxinic acid (I) obtained from the acidic portion.[4] Pyxinic acid was then the first example of the lichen triterpenoid possessing a hydroxyl function at C-3 of the hopane skeleton. Further examination of the neutral fraction obtainable from the ether extract of the same lichen has led us to isolate several triterpenoids with which the present paper is concerned. As schemed in Chart 1, six compounds, named diacetylpyxinol (II), pyxinol (III), 12-desoxy-diacetylpyxinol (IV), 3-O-acetylpyxinol (V), methyl pyxinate (VI), and methyl 3-O-acetyl-pyxinate (VII), have been isolated for the first time, and the structures have been elucidated on the basis of the chemical and physicochemical evidences which are detailed in this paper.

Diacetylpyxinol (II),[5] the major constituent, $C_{34}H_{56}O_6$, mp 240—241°, $[\alpha]_D$ +9.9° ($CHCl_3$), exhibits a positive Liebermann-Burchard color test and is negative to a tetranitromethane test. The infrared (IR) spectrum (in Nujol) of II shows the hydroxyl absorption band at 3420 cm^{-1} and the absorption bands due to the acetoxyl functions at 1735, 1245, and 1235 cm^{-1}. The nuclear magnetic resonance (NMR) spectrum (Table I[6]) of II indicates the presenee of two acetoxyl groups, three hydrogens on the carbons adjacent to the oxygen functions, and eight tertiary methyl groups. Among the tertiary methyls, the chemical shifts of two singlets

1) Part IV: I. Yosioka, T. Nakanishi, M. Yamaki, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 487 (1972).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) *a*) Y. Asahina and S. Shibata, "Chemistry of Lichen Substances," Japan Society for the Promotion of Science, Tokyo, 1954, pp. 34—40; *b*) C.F. Culberson, "Chemical and Botanical Guide to Lichen Products," The University of North Carolina Press, Chapel Hill, 1968, pp. 196—208.
4) *a*) I. Yosioka, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 613; *b*) (+)-Skyrin, an orange bisanthraquinone pigment, has been isolated from the acidic portion. I. Yosioka, K. Morimoto, K. Murata, H. Yamauchi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19** 2420 (1971).
5) I. Yosioka, H. Yamauchi, and I. Kitagawa, *Tetrahedron Letters*, **1969**, 4241 (Preliminary account on the structure of diacetylpyxinol).
6) Assignment of all the C-methyl signals in Table I has been made referring to the following literatures. *a*) J.M. Lehn, *Bull. Soc. Chim. France*, **1962**, 1832; *b*) J.C. Mani, *Ann. Chim.*, **10**, 533 (1965); *c*) H.T. Cheung and D.G. Williamson, *Tetrahedron*, **25**, 119 (1969).

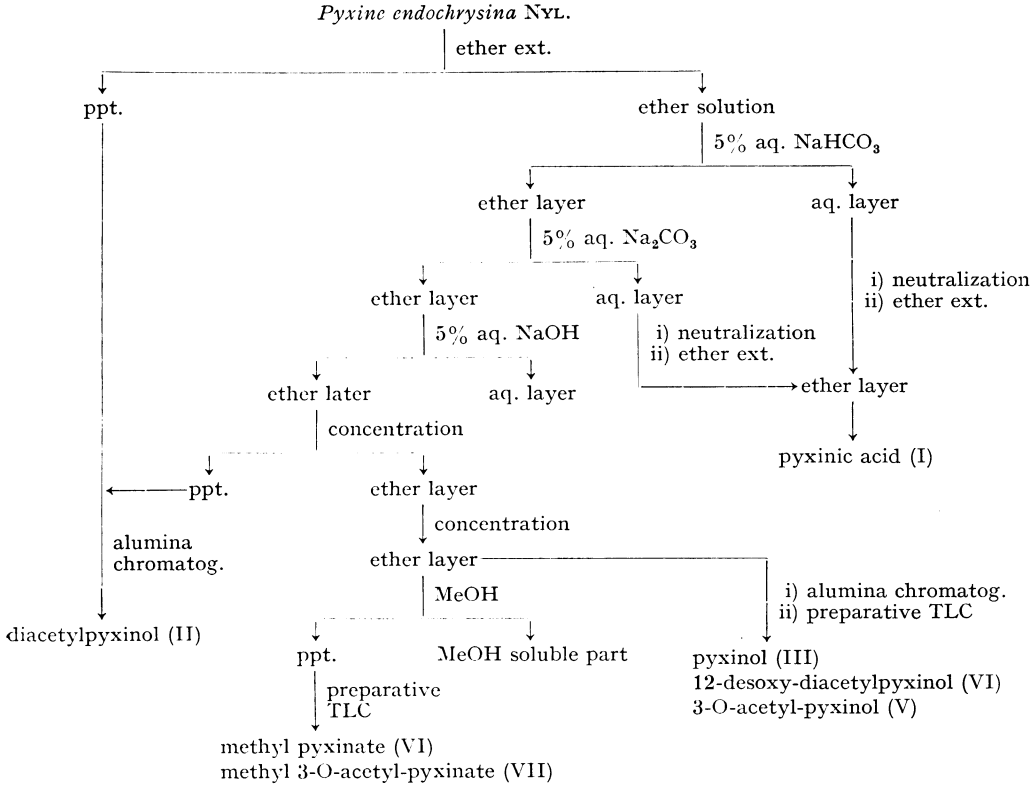


Chart 1

at 8.55 and 8.48 τ (3H each) are in close similarity to the values (8.58—8.52 τ) observed for the methyl protons of the α-acetoxy-isopropyl function in 22-acetoxyhopane derivatives .[7] On treatment with alkali or lithium aluminum hydride, II gave a triol, which was proved identical with natural pyxinol (III), $C_{30}H_{52}O_4$, mp 225—226°, $[\alpha]_D$ +62.8° ($CHCl_3$), isolated as shown in Chart 1. Pyxinol (III) lacks the acetyl function while it possesses a hydrogen on a carbon bearing a hydroxyl function as revealed by a diamagnetically shifted signal[8] at 6.73 τ (5.48 τ in II) and all of the methyl singlets are observed above 8.70 τ in its NMR spectrum.

Acetylation of II under the various conditions furnished an identical triacetate (VIII), $C_{36}H_{58}O_7$, mp 180—181°, which was also prepared by treatment of III with acetic anhydride and *p*-toluenesulfonic acid.[9] The triacetate (VIII), which exhibits three acetoxyl signals at 8.06, 8.00 and 7.99 τ in the NMR spectrum and shows no hydroxyl absorption band in the IR spectrum, regenerated III on alkaline treatment. These observations suggest that diacetylpyxinol (II) is a triterpenoid possessing two acetoxyls (one secondary and one tertiary), one secondary hydroxyl function and an oxygen function presumably of an ether linkage. Treatment of pyxinol (III) with chromium trioxide–pyridine complex afforded a mixture which was separated by column chromatography to give 3-dehydropyxinol (=monoketopyxinol[5]) (IX), $C_{30}H_{50}O_4$, mp 184—185°, and 3,12-didehydropyxinol (=diketopyxinol[5]) (X),

7) S. Huneck and J.M. Lehn, *Bull. Soc. Chim. France*, **1963**, 1702.
8) Due to the overlapping by another multiplet centered at 6.44 τ, the signal is assigned only as a multiplet although it should be a characteristic quartet ascribable to C-3α-H.
9) On acetylation with acetic anhydtide in pyridine at ordinary temperature, II yielded only a small quantiy of VIII.

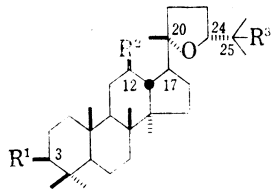
$C_{30}H_{48}O_4$, mp 176—178.5°. These compounds were further converted by the modified Wolff-Kishner reduction[10] to 3-desoxypyxinol (XI), $C_{30}H_{52}O_3$, mp 196.5—197.5° and 3,12-didesoxypyxinol (XII), $C_{30}H_{52}O_2$, mp 138.5—140°, respectively.

In the mass spectra of acetylpyxinol (II), the triacetate (VIII), 3-desoxypyxinol (XI), and 3,12-didesoxypyxinol (XII), the prominent fragment ions appearing at *m/e* 249 (a), and 189 (b) of II and VIII and at *m/e* 191 (c) of XI and XII are presumably ascribed to the rings A and B of the pentacyclic triterpenoids arraying the methyl groups as shown in Chart 2[1,11] and consequently it has been suggested that the only one acetoxyl group of II and VIII locates at either A or B ring. Furthermore, in the NMR spectrum of II, a signal due to a proton geminar to a secondary acetoxyl group is observed at 5.48 τ as a quartet typical X part of an ABX system and the large coupling constant (J=6.7 and 10.0 Hz) defines the acetoxyl function as equatorial and to be adjacent to quaternary carbon on one side and to a methylene group on the other,[12a] thus suggesting the location of the acetoxyl function at C-3β. The assumption is further supported by the following evidence that 3-dehydropyxinol (IX) exhibits a positive Zimmermann color test and positive Cotton effect having the sign and the amplitude comparable to 3-keto-triterpenoid such as oleanan-3-one and lupan-3-one.[13] It is therefore concluded that one secondary acetoxyl group of II locates at C-3β and a partial structure [A] is presented.

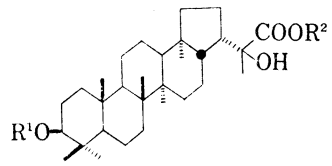
The partial structures in regard to the remaining three oxygen functions of II, which are supposed to locate in the rings other than A and B, have been disclosed as follows. The one secondary hydroxyl group of II is involved in the part structure >CH-CH(OH)-CH_2- and has an equatorial orientation, since the carbinyl proton appears at 6.53 τ as a sextet with the coupling constant of 4.5 Hz and 10.5 Hz in the NMR spectrum of II.[12b] Oxidation of II with chromium trioxide in pyridine furnished 12-dehydro-diacetylpyxinol (XIII), $C_{34}H_{54}O_6$, mp 152—153°, which exhibits a negative Zimmermann color test and shows the existence of a six-membered ring ketone at 1708 cm^{-1} in its IR spectrum. Therefore, the chemical environment of the secondary hydroxyl function is depicted as the partial sturucture [B].

The prediction that II possesses an α-acetoxy-isopropyl partial sturucute [C] is supported by the following reasons: i) Two singlets at 8.55 and 8.48 τ (3H each) of II attributable to the methyls attached to a carbon having an acetoxyl function are found diamagnetically shifted (above 8.70 τ) in the NMR spectrum of III. ii) 3,12-Didesoxypyxinol (XII) exhibits a hydroxyl absorption band at 3580 cm^{-1} in its IR spectrum, while the carbon attached by the hydroxyl function has no hydrogen and all the tertiary methyl signals are observed above 8.77 τ in its NMR spectrum. iii) The hydroxyl function of XII resists acetylation with acetic anhydride in pyridine under the ordianry condition, but is acetylated with acetic anhydride and *p*-toluenesulfonic acid to yield a monoacetate (XIV), $C_{32}H_{54}O_3$, mp 122—123.5°, the NMR spectrum of which shows two methyl signals at 8.52 τ similarly as low as in II. It also supports the above consideration that dehydration of XII with phosphorus oxychloride in pyridine gave an isopropenyl derivative (XV), $C_{30}H_{50}O$, mp 101—103.5°, which was isolated in a pure form by preparative thin-layer chromatography (TLC) using silica gel impregnated with silver nitrate. The yield was fairly low as compared with the case of dehydration of 22-hydroxyhopane derivatives under the same reaction condition.[1,14] In addition, it is worth to mention that the isopropylidene isomer was not obtained in the present case.

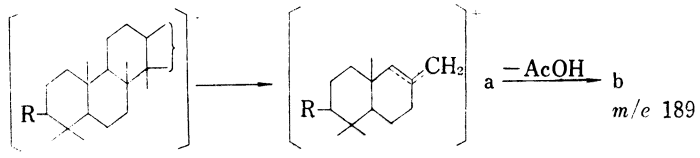
10) W. Nagata and H. Itazaki, *Chem. & Ind.*, **1964**, 1194.
11) *a*) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963); *b*) M.H. Galbraith, C.J. Miller, J.W.L. Rawson, E. Ritchie, J.S. Shannon, and W.C. Taylor, *Aust. J. Chem.*, **18**, 226 (1965); *c*) R.E. Corbett and H. Young, *J. Chem. Soc.* (*C*), **1966**, 1556.
12) *a*) N.S. Bhacca and D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, 1964, pp. 142, 144; *b*) *Idem*, *ibid.*, p. 82.
13) C. Djerassi, J. Osiecki, and W. Closson, *J. Am. Chem. Soc.*, **81**, 4587 (1959).
14) I. Yosioka, T. Nakanishi, H. Yamauchi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 147 (1972), and the literatures described therein.



II : $R^1=R^3=OAc$, $R^2=OH$
diacetylpyxinol
III : $R^1=R^2=R^3=OH$
pyxinol
IV : $R^1=R^3=OAc$, $R^2=H$
12-desoxy-diacetylpyxinol
V : $R^1=OAc$, $R^2=R^3=OH$
3-O-acetyl-pyxinol
Va : $R^1=OAc$, $R^2=O$, $R^3=OH$
XXI : $R^1=\alpha$-OH, $R^2=R^3=OH$
triterpene C



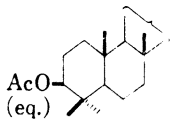
I : $R^1=R^2=H$ pyxinic acid
VI : $R^1=H$, $R^2=CH_3$ methyl pyxinate
VII : $R^1=Ac$, $R^2=CH_3$
methyl 3-O-acetyl-pyxinate



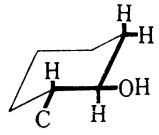
II, VIII : R=OAc
XI, XII : R=H

a: R=OAc *m/e* 249
c: R=H *m/e* 191

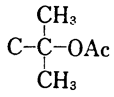
m/e 189



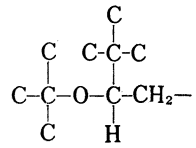
[A]



[B]



[C]



[D]

Chart 2

Finally, as for the ether linkage the partial structure [D] has been forwarded, since only a single proton is concerned in the system as revealed by the NMR signals observed at 6.24 τ (1H, triplet, J=6.0 Hz) in XII and at 6.08 τ (1H, triplet, J=6.4 Hz) in XIV. Accordingly, the partial structures [A], [B], [C], and [D] have now been disclosed for diacetylpyxinol (II).

At the earlier stage of the investigation, we presumed the hopane skeleton for diacetylpyxinol (II), since II carries the above-mentioned partial structures conformable to the hopane skeleton which is the basic carbon framework of coexisting pyxinic acid (I) and has been known to occur fairly often in the lichen family.[3] Therefore some attempts have been made to convert 3,12-didesoxypyxinol (XII) to a hydrocarbon supposedly having a hopane skeleton. The attempted fission of the ether linkage using ethanolic hydrochloric acid, lithium in ethylamine, or lithium aluminum hydride-aluminum chloride was without success. Moreover, dehydration of the tertiary hydroxyl gorup with phosphorus oxychloride in pyridine or thionyl chloride in pyridine was also attempted. However, all the efforts have not given the effective information.[15] Instead, it has become rather inconsistent to account for the following

15) It has become clear after establishment of the structure of diacetylpyxinol that the results are in accord with the finding on gratiogenin. Gratiogenin possessing the same side chain as pyxinol (III) was treated by Tschesche, *et al.* to cleave the ether linkage and dehydrate the tertiary hydroxyl group under the various conditions. However, the results were unsatisfactory. R. Tschesche, G. Biernoth, and G. Snatzke, *Ann.*, **674**, 196 (1964).

results by the use of hopane skeleton. Oxidation of pyxinol (III) with the Kiliani reagent gave a trisnor compound (XVI), $C_{27}H_{40}O_4$, mp 235—237°, the IR spectrum of which indicates the carbonyl absorption bands at 1765 and 1700 cm^{-1}(assignable to γ-lactone and six-membered ring ketone). On oxidation with the same reagent, XII furnished a compound (XVII), $C_{27}H_{44}O_2$, mp 192—195°, the IR spectrum of which (in CCl_4) shows also a carbonyl absorption band at 1775 cm^{-1} assignable to γ-lactone. The NMR spectrum of XVII shows six methyl singlets but lacks a signal attributable to the proton at the carbon bearing the ether linkage. Reduction of XVII with sodium borohydride and subsequent acetylation of the resulted lactol (XVIII), $C_{27}H_{46}O_2$, mp 229—229.5°, with acetic anhydride and *p*-toluenesulfonic acid yielded a lactol acetate (XIX), $C_{29}H_{48}O_3$, mp 123.5—125°.

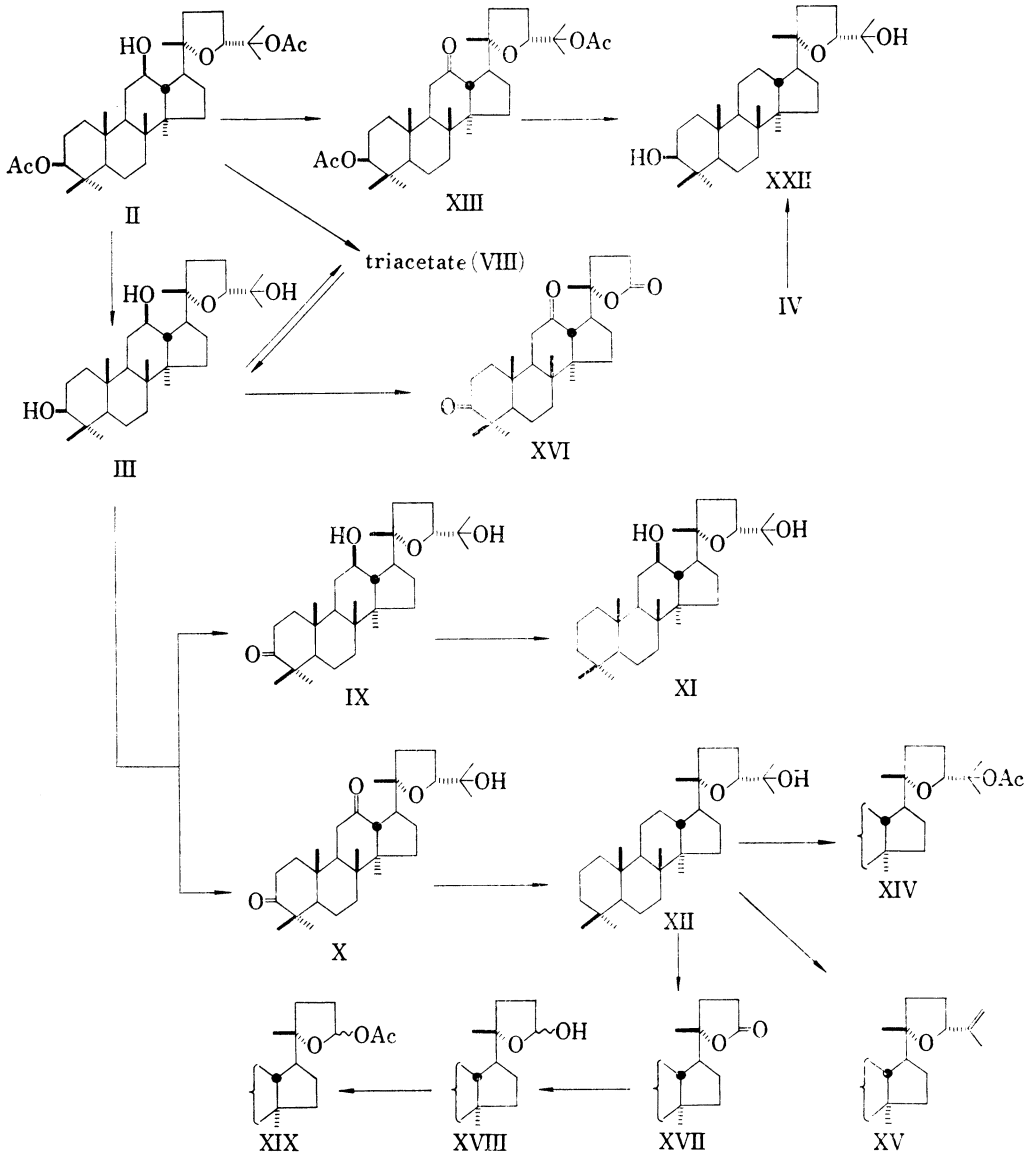


Chart 3

To elucidate the carbon framework and the stereostructure of the pyxinol derivatives, the X-ray crystallographic analysis has been performed using a 3,12-di-O-*p*-bromobenzoate (XX), $C_{44}H_{58}O_6Br_2$, mp 165—168°. The compound (XX) regenerated pyxinol (III) by alkaline hydrolysis in a quantitative yield, thus proving that the former possesses the same carbon skeleton as the latter. Quite interestingly, the bromo-derivative has been disclosed to have a dammarane carbon framework depicted as XX (Chart 4)[16] and as a consequence, the final stereostructure of diacetylpyxinol has now been confimed to be 3*β*,25-diacetoxy-12*β*-hydroxy-20(*S*),24(*R*)-epoxy-dammarane (II). Furthermore, all the derivatives of pyxinol have been rationally formulated by the structures illustrated in Chart 3.

Diacetylpyxinol (II) is the first example of a dammarane triterpenoid discovered in the lichen family, and in addition, it has become clear that pyxinol (III) corresponds to a C-3

TABLE I. The NMR Data given in τ Values[a]

	-CH-O-			OAc	C-CH$_3$						
	C-3	C-12	C-24		C-4α	C-4β	C-10β	C-8β	C-14α	C-20	C-25
II[b]	5.48 (q, 6.7, 10.0)	6.53 (se, 4.5, 10.5)	5.89 (t, 6.4)	7.99 7.99	9.16	9.16	9.13	9.01	9.09	8.76	8.55 8.48
III	6.73 (m)	6.44 (m)	6.13 (t, 7.0)	—	8.99	9.20	9.11	8.99	9.06	8.87	8.70 8.70
IV	5.56 (q, 6.4, 8.6)	—	6.15 (t, 6.4)	8.07 8.00	9.15	9.15	9.15	9.06	9.15	8.91	8.58 8.58
V	5.49 (t. l., $W_{h/2}$=19)	6.47 (se, 4.7, 9.7)	6.14 (t, 6.9)	7.97	9.15	9.15	9.11	9.01	9.11	8.90	8.72 8.72
Va	5.52 (q, 6.4, 8.3)	—	6.30 (t.l.)	7.95	9.11	9.11	9.03	8.78	9.22	8.78	8.88 8.88
VIII	5.48 (m, $W_{h/2}$=18)	5.10 (m)	6.09 (t, 6.0)	8.06 8.00 7.99	9.14	9.14	9.14	9.00	9.06	8.82	8.57 8.57
IX	—	6.46 (se, 9.9, 4.7)	6.13 (t, 6.9)	—	8.93	8.97	9.04	8.97	9.09	8.91	8.72 8.72
X	—	—	6.30 (t. l.)	—	8.89	8.97	8.93	8.75	9.22	8.79	8.89 8.89
XI	—	6.49 (se, 3.9, 9.4)	6.16 (t, 6.6)	—	9.15	9.19	9.15	9.01	9.09	8.91	8.73 8.73
XII	—	—	6.24 (t, 6.0)	—	9.14	9.18	9.14	9.03	9.11	8.77	8.85 8.85
XIII	5.47 (m, $W_{h/2}$=17)	—	6.06 (t, 6.8)	8.00 7.93	9.10	9.10	9.01	8.78	9.21	8.94	8.52 8.50
XIV	—	—	6.08 (t, 6.4)	8.02	9.15	9.19	9.15	9.05	9.12	8.88	8.52 8.52
XV[c]	—	—	5.78 (m)	—	9.17	9.21	9.17	9.06	9.14	8.86	—
XVI	—	—	—	—	8.90	8.95	8.93	8.74	9.21	8.74	—
XVII[d]	—	—	—	—	9.15	9.18	9.15	9.03	9.09	[e]	—
XVII[b]	—	—	—	—	9.17	9.21	9.17	9.06	9.13	8.67	—
XIX	—	—	5.91 (t, 6.0)	7.96	9.15	9.19	9.15	9.03	9.11	8.86	—
XXII	6.72 (q, 6.2, 9.4)	—	6.17 (q, 5.3, 7.7)	—	8.98	9.17	9.10	8.98	9.06	8.74	8.81 8.81

a) Coupling constants in the parentheses are given in Hz, and the following abbreviations are used: m=multiplet, q=quartet, s=singlet, se=sextet, t=triplet, t.l.=triplet like, $W_{h/2}$=half band width in Hz.
b) measured at 100 MHz
c) The compound(XV) shows the signals at 8.33 (3H), 5.30 (1H), and 5.07 τ (1H)(all broad singlets) due to the isopropenyl function.
d) quoted from the literature 6b
e) not given in the literature 6b

16) H. Yamauchi, T. Fujiwara, and K. Tomita, *Tetrahedron Letters*, **1969**, 4245.

epimer of triterpene C (XXI) which was isolated from *Betula platyphylla* SUKATCHEV var. *japonica* HARA and has been recently disclosed by Tanaka, *et al.*[17]

The physicochemical properties of the pyxinol derivatives have given us the useful informations on the chemical structures of the minor constituents. For instance, the mass spectra of the pyxinol derivatives having 20,24-epoxy-25-hydroxyl function show the base peak at *m/e* 143 attributable to an oxonium ion (d) as has been assigned in the mass spectrum of gratiogenin[15] or triterpene C (XXI),[17] while in the mass spectra of the derivatives having 20,24-epoxy-25-acetoxyl function the base peak is observed at *m/e* 125 (f) which is derivable from ion (e) of *m/e* 185 *via* elimination of acetic acid and the process is substantiated by the presence of a metastable ion peak at *m/e* 84.3 (calcd. *m/e* 84.4).

The second major constituent (V), $C_{32}H_{54}O_5$, mp 196—197°, $[\alpha]_D$ +28.2° ($CHCl_3$), exhibits the presence of the hydroxyl and acetoxyl functions in the IR spectrum. The NMR spectrum (Table I) indicates the presence of one acetoxyl group, eight tertiary methyl singlets appearing above 8.72 τ, and three protons attached to the carbons adjacent to the oxygen functions. Among the latter, the triplet-like signal at 5.49 τ suggests the presence of an equatorial acetoxyl function at C-3. Furthermore, the mass spectrum of V giving a base peak at *m/e* 143 (d) demonstrates the presence of the same side chain as that of pyxinol (III). On chromium trioxide–pyridine complex oxidation, V furnished a monoketone (Va) which is negative to the Zimmermann test. In conclusion, V has been assumed to be 3-O-acetyl-pyxinol and identified with the authentic sample prepared by acetylation of pyxinol (III) with acetic anhydride in pyridine at ordinary temperature. It is noticeable to point out here that two hydroxyl functions at C-3 and C-12 of pyxinol (III) exhibit different reactivity towards acetylation. The C-12 hydroxyl was unaffected by ordinary acetylation, which would be ascribed to the sterical congestion caused by the bulky group at C-17. The intramolecular hydrogen bonding between C-12-OH and the ethereal oxygen observed in the IR spectra of II (at 3432 cm^{-1}) and XI (at 3430 cm^{-1}) would corroborate the assumption.

12-Desoxy-diacetylpyxinol (IV), $C_{34}H_{56}O_5$, mp 190—193°, $[\alpha]_D$ +37.5° ($CHCl_3$), exhibits the acetoxyl absorption bands at 1735 and 1245 cm^{-1} but no hydroxyl band in the IR spectrum. The NMR spectrum (Table I) shows eight tertiary methyl signals, of which a singlet at 8.58 τ (6H) suggests the presence of an α-acetoxyisopropyl group, and shows the signals at 5.56 τ (1H, quartet, J=6.4 & 8.6 Hz) and 6.15 τ (1H, triplet, J=6.4 Hz) due to the protons on the carbon atoms bearing the oxygen functions. These signals are comparable to the signals due to the protons at C-3 and C-24 of II. Consequently, IV has been assumed to be 12-desoxy-diacetylpyxinol and the assumption was verified by the fact that alkaline hydrolysis of IV yielded a compound being identical by mixed mp, IR ($CHCl_3$), and TLC with 12-desoxypyxinol (XXII) obtained by the Huang-Minlon reduction of XIII.

It is quite interesting from the chemotaxonomical and biogenetic viewpoints that several triterpenoids having analogous patterns of hydroxylation and acetylation are found in the same lichen as was previously encountered in a lichen *Parmelia entotheiochroa* HUE., which contains zeorin (XXIII) and leucotylin (XXIV), and their analogues.[18]

On the other hand, Tanaka and his co-workers have proposed[17] that the tetrahydofuran derivatives (having the partial structure (h)) might be the artifacts probably induced secondarily from their corresponding hydroxy-epoxide precursors (partial structure (g)) during the process of extraction or separation, since the epoxide of this type has been recognized to be converted readily to the corresponding tetrahydrofuran derivative.[15,19] Taking account of the fact that the lichen mentioned here contains the compounds possessing the acetoxyl function

17) M. Nagai, N. Tanaka, S. Ichikawa, and O. Tanaka, *Tetrahedron Letters*, **1968**, 4239.
18) I. Yosioka, M. Yamaki, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 804 (1966).
19) *a*) J.F. Biellmann, *Tetrahedron Letters*, **1966**, 4803; *b*) E.W. Warnhoff and C.M.M. Halls, *Can. J. Chem.*, **43**, 3311 (1965).

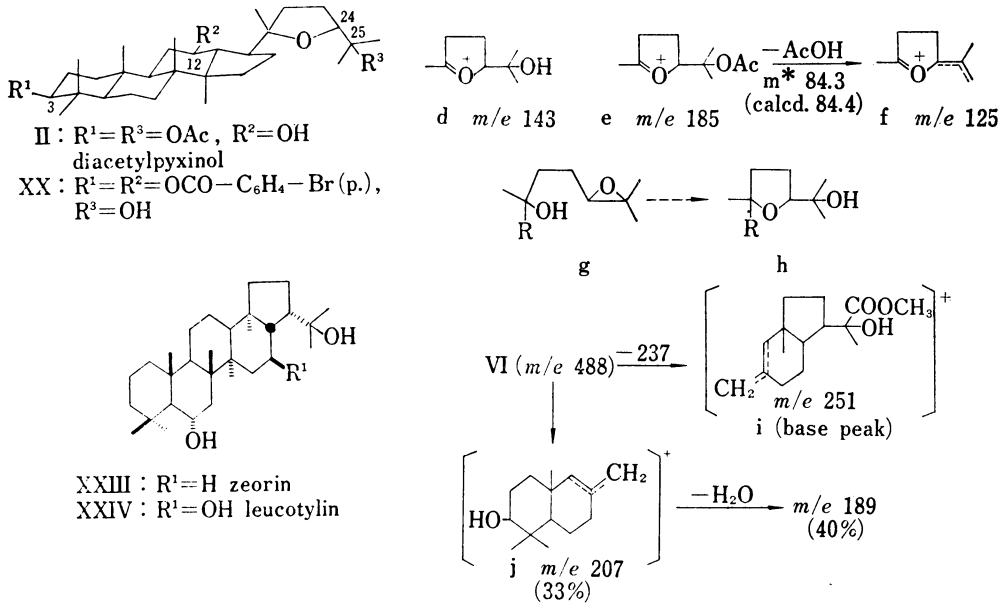


Chart 4

at C-25, the natural occurrence of the pyxinol derivatives seems to be most likely.

Finally, the isolation of methyl pyxinate (VI),[4a] mp 282—284° $[\alpha]_D$+39.5° ($CHCl_3$), and methyl 3-O-acetyl-pyxinate (VII),[4a] mp 255—255.5°, $[\alpha]_D$ +39.2° ($CHCl_3$), was accomplished in the manner as schemed in Chart 1. The IR spectrum of VI indicates the presence of the carbonyl absorption band at 1713 cm^{-1}. The mass spectrum (Chart 4) exhibits the molecular ion peak at *m/e* 488 and the base peak at *m/e* 251 (i), which has not been observed among the pyxinol derivatives. In addition, the total fragmentation pattern is quite resembled to that of leucotylin and leucotylic acid derivatives,[1] so that the similar pentacyclic structure has been presumed for VI. Based on these evidences as well as the coexistence with pyxinic acid (I) in the same lichen, VI has been assumed to be methyl pyxinate and the assumption was verified by the direct comparison with the authentic sample.[4a]

The compound (VII) shows the hydroxyl absorption band at 3450 cm^{-1} and the carbonyl bands at 1728 and 1695 cm^{-1} in its IR spectrum. The mass spectrum exhibits the molecular ion peak at *m/e* 530 and the base peak at *m/e* 251 (i), the latter being identical with that of VI. The prominent fragment ions appearing at *m/e* 249 and 189 suggest that there is an acetoxyl function attached to the ring A or B in its molecule. In conclusion, VII was assumed to be methyl 3-O-acetyl-pyxinate and identified with the authentic sample[4a] by mixed mp, IR (KBr) and TLC.

It has been elucidated that the dammarane type and the hopane type triterpenoids are occurring together in the same lichen *Pyxine endochrysina* Nyl. The similar coexistence has already been revealed in the higher plant origins such as dammar resin (Dipterocarpaceae)[20] and the leaves of Japanese white birch (Betulaceae).[17] In this connection, the transformation of hydroxyhopanone into a dammarane type derivative accomplished by Fujimoto and Tanaka[21] seems to be of interest.

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Experimental[22]

Isolation of Triterpenoids——The air dried lichen *Pyxine endochrysina* NYL. (1.78 kg) collected at Misakubo in Shizuoka prefecture was crushed and extracted with ether (10 liter) at room temperature successively. During the period the pale yellow crystals (25 g) were precipitated. The combined ether solution was partially concentrated and extracted with aq. 5% $NaHCO_3$, 5% Na_2CO_3, and 5% NaOH successively as shown in Chart 1. From the parts soluble in aq. $NaHCO_3$ and Na_2CO_3, pyxinic acid (I)[4a] (1.5 g, 0.08% from the starting lichen) was obtained.

Since the above collected pale yellow precipitates (25 g) were shown by TLC to contain the identical constituents with the precipitates obtained by the concentration of the neutral ether fraction, the both precipitates were combined, washed with ether and chromatographed on alumina (Woelm grade I) eluting with benzene to give diacetylpyxinol (II) (colorless needles by recrystallization with EtOAc, 7.9 g, 0.44% from the lichen). The analytical sample recrystallized with EtOAc melted at 240—241°, $[\alpha]_D$ +10.0° (*c*, 1.0). *Anal.* Calcd. for $C_{34}H_{56}O_6$: C, 72.82; H, 10.06. Found: C, 72.71; H, 9.90. IR ν_{max}^{Nujol} cm^{-1}: 3420 (OH), 1730, 1245, 1235 ($OCOCH_3$), 1020 (C-O-C), IR $\nu_{max}^{CCl_4}$ (0.5×10^{-3} mole) cm^{-1}: 3640, 3432 (OH). NMR (100 MHz) τ: 4.92 (1H, broad singlet, OH, disappeared by D_2O addition) and signals as tabulated in Table I.

Further concentration of the neutral ether soluble portion gave a yellow resinous residue (7.3 g), which was chromatographed on neutral alumina (Sumitomo) eluting with benzene, benzene-$CHCl_3$ (3:1), $CHCl_3$, and MeOH successively. The residue obtained by evaporation of the benzene eluate was purified by preparative TLC to give 12-desoxy-diacetylpyxinol (IV), mp 190—193° (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D$ +37.5° (c, 1.02). *Anal.* Calcd. for $C_{34}H_{56}O_5$: C, 74.95; H, 10.36. Found: C, 75.11; H, 10.30. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1245 ($OCOCH_3$). Mass Spectrum *m/e* (%): 529 (1), 484 (41), 383 (34), 249 (4), 189 (47), 185 (89), 125 (100).

The earlier $CHCl_3$ eluate was concentrated and chromatographed again using silicic acid (Mallinckrodt). Elution with benzene–$CHCl_3$ (1:1) followed by recrystallization with aq. MeOH gave 3-O-acetyl-pyxinol (V), mp 196—197°, $[\alpha]_D$ +28.2° (*c*, 1.06). *Anal.* Calcd. for $C_{32}H_{54}O_5$: C, 74.09; H, 10.49. Found: C, 73.82; H, 10.48. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (sh) (OH), 1730, 1245 ($OCOCH_3$). Mass Spectrum *m/e* (%): 503 (3), 500 (3), 485 (3), 482 (3), 459 (4), 249 (7), 189 (25), 143 (100).

The later $CHCl_3$ eluate gave crude material, which was chromatographed again on neutral alumina (Sumitomo) followed by preparative TLC (Merck silica gel, $CHCl_3$–MeOH (60:1)) giving pyxinol (III) (cololress needles from aq. MeOH), mp 225—226°, $[\alpha]_D$ +62.8° (c, 0.99). *Anal.* Calcd. for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00. Found: C, 75.79; H, 10.80. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3430 (OH). Mass Spectrum *m/e* (%): 463 (1), 417 (4), 400 (68), 381 (27), 207 (50), 189 (25), 143 (100).

The precipitates obtained by addition of MeOH to the oily neutral ether soluble portion were chromatographed on a column of silicic acid (Mallinckrodt). Elution with benzene gave crude VI and VII successively. The crude VI was further purified by preparative TLC followed by crystallization from MeOH giving a pure sample of methyl pyxinate (VI), mp 282—284° (colorless crystals), $[\alpha]_D$ +39.5° (*c*, 0.88). IR ν_{max}^{KBr} cm^{-1}: 1713 (br), 1250 ($COOCH_3$). Mass Spectrum *m/e* (%): 488 (2), 470 (2), 455 (3), 437 (3), 429 (3), 385 (14), 367 (15), 251 (100), 233 (39), 207 (33), 189 (40). The crude VII was also purified by preparative TLC followed by crystallization from aq. MeOH to give methyl 3-O-acetyl-pyxinate (VII), mp 255—255.5° (colorless needles), $[\alpha]_D$ +39.2° (*c*, 1.0). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1728, 1695, 1250 ($OCOCH_3$, $COOCH_3$). Mass Spectrum *m/e* (%): 530 (6), 515 (1), 470 (6), 455 (7), 437 (4), 428 (19), 411 (5), 367 (28), 251 (100), 249 (11), 233 (33), 189 (51).

Pyxinol (III)——i) A solution of diacetylpyxinol (II) (1.35 g) in 5% KOH–MeOH (180 ml) was refluxed for 3 hr. During the period crystals were separated out. The total reaction mixture was poured into ice-water containing dil. HCl, and the crystals were collected by filtration and recrystallized with aq. MeOH to give III (colorless needles, 1.08 g). ii) Diacetylpyxinol (II) (1.50 g) in dry ether (100 ml) was treated under reflux with an excess of $LiAlH_4$ (700 mg) for 7 hr. After cooling, the reaction mixture was treated with aq. ether, aq. 5% H_2SO_4 solution, and extracted with ether repeatedly. The combined ethereal extracts were washed with water, dried over $MgSO_4$ and evaporated to dryness. The crystalline residue (1.26 g) was then recrystallized with aq. MeOH giving a product, which was identified with III by mixed mp, IR (KBr) and TLC.

22) Melting points were taken on the Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and are uncorrected. Optical rotations were measured in $CHCl_3$ with the Rex Photoelectric Polarimeter NEP-2. IR spectra were obtained with the Hitachi Infrared Spectrophotometer EPI-S2 and EPI-2, Mass spectra were taken on the Hitachi RMU-6D Mass Spectrometer. Unless stated otherwise, NMR spectra were recorded at 60 MHz on the Hitachi Perkin-Elmer H-60 Spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard. Optical rotatory dispersion (ORD) curves were taken on the JASCO ORD/UV-5 Automatic Recording Spectropolarimeter. Preparative TLC separation was performed using Camag silica gel unless stated otherwise.

Preparation of Triacetate (VIII)——i) A mixture of II (202 mg) and fused NaOAc (100 mg) in Ac_2O (4 ml) was heated under reflux for 2 hr. The solid residue obtained after working up in the usual manner was repeatedly recrystallized with *n*-hexane, and then with aq. MeOH to give the triacetate (VIII) (colorless needles), mp 180—181°. *Anal.* Calcd. for $C_{36}H_{58}O_7$: C, 71.72; H, 9.70. Found: C, 71.79; H, 9.78. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1245 ($OCOCH_3$). Mass Spectrum *m/e* (%): 587 (0.2), 542 (2), 501 (3), 249 (2), 189 (8), 185 (49), 125 (100).

ii) A suspension of II (620 mg) and *p*-TsOH·H_2O (10 mg) in Ac_2O (42 ml) was stirred for 2 hr at room temperature to give a solution which was then let stand at 28°. After 16 hr the solution was poured into ice-water and extracted with ether. The combined ether solution was washed with aq. 5% Na_2CO_3, water, dried over $MgSO_4$ and evaporated. Colorless needles (467 mg) obtained by recrystallization of the residue with aq. MeOH was identified by mixed mp, IR (CCl_4), and TLC with the above described acetylation product (VIII).

iii) A suspension of pyxinol (III) (895 mg) and *p*-TsOH·H_2O (10 mg) in Ac_2O (30 ml) was dissolved with stirring for 2 hr at room temperature, and then let stand for 20 hr at 28°. After working up in the usual way, the crystalline product was chromatographed over silicic acid (Mallinckrodt) with the aid of benzene–$CHCl_3$ mixture. Elution with benzene–$CHCl_3$ (2:3) followed by recrystallization with aq. MeOH gave colorless needles of VIII (982 mg).

Alkaline Hydrolysis of Triacetate (VIII) yielding Pyxinol (III)——Triacetate (VIII) (14 mg) was hydrolyzed with 5% KOH–MeOH (5.8 ml) under reflux for 6 hr. The hydrolysate obtained after the usual work up yielded pyxinol (III) (11 mg, recrystallized with aq. MeOH) identified by mixed mp, IR (KBr), and TLC.

Oxidation of Pyxinol (III) giving 3-Dehydropyxinol (IX) and 3,12-Didehydropyxinol (X)——To a suspension of CrO_3–pyridine complex (CrO_3 4.0 g and pyridine 70 ml) was added a solution of pyxinol (6.80 g) in pyridine (80 ml). The total mixture was stirred for 3.5 hr at room temperature and then kept at 30°. After 15.5 hr excess CrO_3 was consumed by addition of a few drops of MeOH and the mixture was added with ether (200 ml) and filtered. The filtrate was washed with water, dried, and evaporated giving a pale yellow oily residue (6.68 g), which was revealed by TLC to contain three components including III. The product was chromatographed on silicic acid (Mallinckrodt) developing with $CHCl_3$. The earlier eluate gave 2.96 g of 3-dehydropyxinol (IX), mp 184—185° (colorless rods from aq. MeOH). *Anal.* Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.94; H, 10.40. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3425 (sh) (OH), 1705 (CO). NMR ($CDCl_3$) τ: as given in Table I; NMR (benzene) τ: 9.29, 9.27, 9.10, 9.06, 8.94, 8.91, 8.88, 8.58 (3H each, all s, $8\times CH_3$). ORD ($c=1.51$, MeOH) [Φ] (nm): +94° (700), +141° (589), +2010° (305) (peak), −534° (272) (trough), +1351° (220). Zimmermann test: positive. Oleanan-3-one[13]: ORD ($c=0.1$, dioxane) [Φ] (nm): −111° (700), +149° (589), +1943° (310) (peak), +656° (282.5). Lupan-3-one[13]: ORD ($c=0.1$, dioxane) [Φ] (nm): +17° (700), +21° (589), +1542° (317.5) (peak), −1133° (280).

From the later eluate was obtained 3,12-didehydropyxinol (X, 2.00 g, colorless needles from aq. MeOH), mp 176—178.5°. *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 75.94; H, 10.26. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3570, 3400 (OH), 1710 (CO). NMR ($CDCl_3$) τ: as given in Table I; NMR (benzene) τ: 9.37, 9.34 (3H each), 9.06 (6H), 8.98, 8.87, 8.83, 8.68 (3H each) (all s, totally eight methyls). Zimmermann test: positive.

Kiliani Oxidation of Pyxinol (III) giving XVI——To a solution of III (981 mg) in acetone (60 ml), 2.5 ml of the Kiliani reagent (composition: CrO_3 2.66 g, conc. H_2SO_4 2.3 ml, water 7.7 ml) was added dropwise with stirring at 0°. After stirring for additional 25 min, the reaction mixture was diluted with ice-water, extracted with ether and worked up in the usual way to give a solid residue (821 mg), which was chromatographed on silica gel (Merck) followed by preparative TLC (Merck, silica gel) to give 3,12-didehydropyxinol trisnorlactone (XVI) (481 mg), mp 235—237° (colorless needles from aq. MeOH). *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.29; H, 9.24. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (γ-lactone), 1700 (CO).

3,25-Di-O-acetyl-12-dehydropyxinol (XIII) (=12-Dehydro-diacetylpyxinol)——i) A solution of II (610 mg) in pyridine (10 ml) was added to a mixture of CrO_3 (710 mg) in pyridine (10 ml) and the reaction mixture was allowed to stand overnight at room temperature. The product obtained after working up in the usual way was chromatographed on alumina (Sumitomo) eluting with $CHCl_3$ to give a product which exhibited the negative Zimmermann color test and was recrystallized with *n*-hexane to give XIII of mp 152—153°, $[\alpha]_D$ +60.3° (*c*, 0.99). *Anal.* Calcd. for $C_{34}H_{54}O_6$: C, 73.08; H, 9.74. Found: C, 73.18; H, 9.94. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1240 ($OCOCH_3$), 1708 (CO). ORD ($c=1.06$, MeOH) [Φ] (nm): +13° (700), +17° (589), +51° (338), 0° (309) (trough), +339° (260) (peak), +325° (250), +492° (220).

ii) A solution of II (6.05 g) in acetone (300 ml) was oxidized at 0° with the Kiliani mixture (10 ml). The product after working up in the usual way followed by recrystallization with *n*-hexane gave a compound (5.81 g) which was identified by IR (CCl_4) and TLC with XIII obtained above.

3-Desoxypyxinol (XI)——A solution of 3-dehydropyxinol (IX) (2.86 g) in EtOH (5 ml) was added with triethylenegylcol (170 g), 80% hydrazine hydrate (25 g), and hydrazine dihydrochloride (5.2 g). The total mixture was refluxed for 4 hr in an oil bath (temp. 150—160°) and added with KOH (11 g). After setting the condenser downward, the bath temperature was raised up to 230—240° gradually to distill of unreacted hydrazine and water completely. The downward condenser was then replaced by an air-cooler and the mixture was heated in the oil-bath (230—240°) for 3 hr. The reaction mixture was cooled, poured into ice-

water, extracted with ether, and worked up in the usual way. The product (2.0 g) was crystallized from aq. MeOH giving XI (colorless needles), mp 196.5—197.5°. *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found: C, 78.43; H, 11.22. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (OH). IR $\nu_{max}^{CCl_4}$ (0.5×10^{-3} mole) cm^{-1}: 3430, 3350 (OH). Mass Spectrum *m/e* (%): 460 (1), 445 (1), 427 (3), 401 (2), 191 (31), 143 (100).

3,12-Didesoxypyxinol (XII)——A solution of 3,12-didehydropyxinol (X) (2.52 g) in EtOH (6 ml) was added with triethyleneglycol (124 g) and freshly redistilled hydrazine (bp 114—115°) and the total mixture was refluxed for 6 hr at 180° (bath temperature). After setting the condenser downward, the reaction mixture was added with KOH (7 g) and the temperature was raised up to 230—240° gradually to distill off unreacted hydrazine and water completely. After refluxing for further 2 hr and 45 min at 230—240°, the reaction mixture was cooled and poured into ice-water. After working up in the usual way, the product was chromatographed on silicic acid (Mallinckrodt). Benzene–$CHCl_3$ (3:2) mixture eluted 3,12-didesoxypyxinol (XII, 1.61 g), mp 138.5—140° (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D$ +84.4° (c, 1.00). *Anal.* Calcd. for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 81.32; H, 11.57. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH). Mass Spectrum *m/e* (%): 429 (3), 411 (1), 385 (19), 367 (2), 191 (14), 143 (100).

Acetylation of 3,12-Didesoxypyxinol (XII) giving XIV——i) A suspension of XII (291 mg) and *p*-TsOH·H_2O (10 mg) in Ac_2O (30 ml) was dissolved with stirring for 2 hr at room temperature and then kept to stand at 28°. After 16 hr the solution was poured into ice-water and extracted with ether. The product was contaminated with small amount of XII, which was removed by preparative TLC and the final product was recrystallized with $CHCl_3$–MeOH to give 3,12-didesoxy-25-O-acetyl-pyxinol (XIV) (227 mg) (colorless scaly crystals), mp 122—123.5°. *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 79.00; H, 11.00. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1730, 1260, 1245 ($OCOCH_3$). Mass Spectrum *m/e* (%): 471 (2), 426 (29), 411 (4), 385 (11), 191 (47), 185 (35), 125 (100).

ii) Acetylation of XII (2 mg) in pyridine (20 drops) and Ac_2O (8 drops) at 27° for 14 hr recovered the starting material (XII). Acetylation for further 24 hr gave only the trace amount of XIV as revealed by TLC.

Dehydration of 3,12-Didesoxypyxinol (XII) with $POCl_3$ yielding Isopropenyl Derivative (XV)——3,12-Didesoxypyxinol (XII) (339 mg) in pyridine (14 ml) was treated with $POCl_3$ (2 ml) for 16.5 hr under ice-cooling. The solution was poured dropwise into ice-water and extracted with ether. After working up in the usual way the product was separated by preparative TLC using silica gel (Camag) impregnated with silver nitrate and crystallized from aq. MeOH to give an isopropenyl compound (XV) (40 mg), mp 101—103.5°. High Resolution Mass Spectrum *m/e*: Calcd. for $C_{30}H_{50}O$ 426.3861. Found: 426. 3879. Mass Spectrum *m/e*: 125 (base peak) (f). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 897 (>C=CH_2).

Kiliani Oxidation of 3,12-Didesoxypyxinol (XII)——3,12-Didesoxypyxinol (XII) (100 mg) in acetone (8 ml) was treated with the Kiliani mixture (8 drops) at 0° for 40 min. The product obtained after usual treatment was recrystallized with $CHCl_3$–MeOH to yield 3,12-didesoxypyxinol trisnorlactone (XVII) as colorless needles (85 mg), mp 192—195°, $[\alpha]_D$ +40.2° (c, 0.73). *Anal.* Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.91; H, 11.13. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1775 (γ-lactone); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760. ORD (*c*=0.73, MeOH) [Φ] (nm): +8° (700), +20° (589), +67° (350), +134° (250). Mass Spectrum *m/e* (%): 400 (10), 385 (6), 301 (11), 191 (100), 99 (55).

Reduction of 3,12-Didesoxypyxinol Trisnorlactone (XVII) with $NaBH_4$——To a solution of XVII (107 mg) in EtOH (20 ml), a solution of $NaBH_4$ (650 mg) in EtOH (20 ml) was added and the mixture was stirred for 4 hr at room temperature and let stand overnight. The solution was poured into dil. AcOH and the crystalline product was repeatedly recrystallized with benzene yielding 3,12-didesoxypyxinol trisnorlactol (XVIII) as colorless needles (107 mg), mp 229—229.5°. *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 81.10; H, 11.96. IR ν_{max}^{Nujol} cm^{-1}: 3280, 3210 (OH).

Acetylation of 3,12-Didesoxypyxinol Trisnorlactol (XVIII) giving XIX——To a solution of lactol (XVIII) (59 mg) in pyridine (7.5 ml) was added dropwise Ac_2O (3 ml) and the total solution was kept at 30° for 17 hr. After working up in the usual way the crude ctystals were chromatographed on silicic acid (Mallinckrodt) developing with benzene and benzene–$CHCl_3$ mixture. Benzene–$CHCl_3$ (10:1) mixture eluted a lactol acetate (XIX), mp 123.5—125° (49 mg, colorless granules from aq. MeOH). *Anal.* Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.43; H, 11.23. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1245 ($OCOCH_3$), 1200 (C-O-C).

***p*-Bromobenzoylation of Pyxinol (III) giving XX**——To a solution of III (196 mg) in pyridine (6 ml) was added dropwise *p*-bromobenzoyl chloride (0.8 ml) under ice-cooling. After keeping at 26° for 15 hr, the reaction mixture was poured into ice-water and extracted with ether. The ether extract, after treating in the usual manner, was found to consist of three compounds (by TLC) and the mixture was separated by preparative TLC developing with $CHCl_3$–benzene (1:1) mixture. The slower moving portion gave the 3,12-di-O-*p*-bromobenzoate, which was recrystallized with $CHCl_3$–EtOH yielding colorless needles (64 mg) of XX, mp 165—168°, exhibiting the positive Beilstein test. *Anal.* Calcd. for $C_{44}H_{58}O_6Br_2$: C, 62.71; H, 6.94; Br, 18.97. Found: C, 62.78; H, 6.88; Br, 19.08. IR ν_{max}^{KBr} cm^{-1}: 3403 (OH), 1711 (CO), 1597 (benzene ring), 1267 (C-O-C). NMR (100 MHz) τ: 9.07 (6H), 9.01, 8.99, 8.97, 8.93, 8.89, 8.78 (3H each) (all s, totally eight methyls), 6.45 (1H, triplet like, >$C_{(24)}$H-O-), 5.28 (1H, diffused multiplet, >$C_{(3)}$H-O-*p*-bromobenzoyl), 4.89 (1H, diffused multiplet, -$C_{(12)}$H-O-*p*-bromobenzoyl), 2.50, 2.18 (4H, A_2B_2 quartet, J=8.0 Hz), 2.47, 2.18 (4H, A_2B_2 quartet, J=8.0 Hz) (aromatic protons).

Alkaline Hydrolysis of 3,12-Di-O-*p*-bromobenzoate (XX)——A solution of XX (35 mg) in 10% KOH–MeOH (50 ml) was refluxed for 4 hr, worked up in the usual manner. The product (8 mg), obtained by recrystallization with aq. MeOH, showed mp 221—223°, undepressed on admixture with pyxinol (III) and exhibited the identical IR spectrum and TLC behavior with III.

Acetylation of Pyxinol (III) giving 3-O-Acetyl-pyxinol (V)——A mixture of III (1.28 g), pyridine (13 ml), and Ac_2O (5.5 ml) was kept to stand at 24° for 15 hr and worked up in the usual way. The product (1.35 g) was recrystallized with aq. MeOH as colorless needles and was identified by IR and TLC with natural 3-O-acetyl-pyxinol (V). No depression on mixed mp determination was also observed.

Oxidation of 3-O-Acetyl-pyxinol (V) giving 3-O-Acetyl-12-dehydropyxinol (Va)——To a mixture of CrO_3 (200 mg) in pyridine (10 ml) was added a solution of V (130 mg) in pyridine (4 ml) and the total mixture was kept at 23° for 2 days. The oily product obtained after usual treatment was chromatographed on silicic acid (Mallinckrodt) developing with benzene. The product was then washed with *n*-hexane and recrystallized with benzene–*n*-hexane mixture to give the ketone (Va) as colorless needles, mp 213—213.5°. *Anal.* Calcd. for $C_{32}H_{52}O_5$: C, 74.37; H, 10.14. Found: C, 74.40; H, 9.99. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1718, 1250 (CO, $OCOCH_3$). Zimmermann test: negative.

Huang-Minlon Reduction of 12-Dehydro-diacetylpyxinol (XIII) to give 12-Desoxypyxinol (XXII)——A solution of 12-dehydro-diacetylpyxinol (XIII) (100 mg) in diethyleneglycol (27 ml) was added with 80% hydrazine hydrate (2.7 ml) and KOH (3.8 mg) and refluxed in an oil bath (bath temperature 165—170°) for 3.5 hr. After treating in the usual manner as for the synthesis of XI from IX, the reaction mixture was poured into ice–water and extracted with benzene. The benzene solution was washed with water, dried, concentrated, and chromatographed on silicic acid (Mallinckrodt). The benzene eluate gave pure 12-desoxy-pyxinol (XXII) (31 mg, colorless scaly crystals by recrystallization with aq. MeOH), mp 197—198°, $[\alpha]_D$ +30.4° (*c*, 1.01). *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found: C, 78.08; H, 11.21. IR ν_{max}^{Nujol} cm^{-1}: 3320 (OH), 1085, 1040 (C-O-C). Mass Spectrum *m/e* (%): 445 (2), 427 (1), 401 (13), 383 (12), 207 (5), 191 (11), 189 (3), 143 (100).

Alkaline Hydrolysis of 12-Desoxy-diacetylpyxinol (IV)——12-Desoxy-diacetylpyxinol (IV) (40 mg) was hydrolyzed with 5% KOH–MeOH (40 ml) by refluxing for 2 hr. After the reaction mixture was treated in the usual way, crystallization of the product from aq. MeOH gave a pure product (30 mg) as colorless plates, which were identified by mixed mp, IR ($CHCl_3$), and TLC with 12-desoxypyxinol (XXII).

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