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DUSTANIN:
A NEW PENTACYCLIC TRITERPENOID AS A FUNGAL METABOLITE

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SEVERAL years ago Dr. Y. Yamamoto at Kanazawa University isolated white crystals of mp. 263-265° from light petroleum extract of mycelia of an Aspergillus fungus (DH-101) which is distributed on dusts in air. The compound gives a positive Liebermann test indicating it to be a steroid or triterpenoid. The structure of this compound, however, was awaited to be investigated until we started the structural studies. In this communication we propose the name, dustanin, to it and represent its structure by the formulation (I).

Dustanin (I), mp. 263-265°, $[\alpha]_D$+20°*, gave a negative test to TNM, and was analysed as $C_{30}H_{52}O_2$. The UV spectrum of the compound was transparent above 210mμ and the IR spectrum showed the presence of hydroxyl groups by the strong absorption at 3390cm^{-1}. Its n.m.r. spectrum exhibited eight methyl groups at 8.82(6H), 8.95, 9.01, 9.16,

* Optical rotations were measured in chloroform solution, UV spectra in cyclohexane, IR spectra in Nujol mulls, and NMR spectra in $CDCl_3$.

9.17, 9.22, and 9.24τ, but no olefinic proton signal. Hence the compound is a pentacyclic saturated triterpenoid diol.

Acetylation of dustanin (I) with acetic anhydride and pyridine gave a monoacetate (II), mp. 203-205°, $[\alpha]_D$-11°, $C_{32}H_{54}O_3$; IR 3390 (OH), 1715 and 1244cm^{-1} (OAc); N.m.r. 8.03τ(3H, OAc). Oxidation of I with chromium trioxide and pyridine yielded a hydroxy-ketone, dustaninone (III), mp. 291°, $[\alpha]_D$-63°, $C_{30}H_{50}O_2$; IR 3448 (OH) and 1678cm^{-1} (CO); negative C. e., $[\phi]_{326m\mu}$-4870°; N.m.r. eight methyls at 8.81 (9H), 8.93, 9.02, 9.14, and 9.18τ(6H). These evidences define the diol function as a secondary-tertiary. That the tertiary hydroxyl group constitutes $-C(OH)(CH_3)_2$ grouping was suggested by the n.m.r. spectra of I, II, and III in which two methyl groups appeared at ca. 8.8τ(8.82 in I, 8.82 and 8.85 in II, 8.81 in III), and confirmed by easy hydrogenolysis of I over platinum oxide in acetic acid and hydrogen to deoxydustanin (IV), mp. 193-195°, $[\alpha]_D$-6°, $C_{30}H_{52}O$. In the n.m.r. spectrum of IV all methyls appeared above 8.9τ and the highest peak corresponding to 3/2H at 9.34τ is obviously a part of coupled methyl group due to the formation of isopropyl function. Oxidation of IV gave deoxydustaninone (V), mp. 207-208°, $C_{30}H_{50}O$; IR 1695cm^{-1} (CO); ORD, negative C. e., $[\phi]_{326m\mu}$-5710°.

When dustaninone (III) was reduced by modified Wolff-Kishner method[1] it produced a hydrocarbon which after purification showed mp. 180-183°. VPC* revealed that this hydrocarbon consisted of about 90% hop-17(21)-ene(hopene-I)[2] (VI). The similar reduction of deoxydustaninone (V) afforded a saturated hydrocarbon (VII), mp. 179-182° which was identical

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* Vapor phase chromatographies were carried out by using 1% SE-30 column, column temp. 230°, carrier gas, Ar.

with zeorinane* as shown by mp., mixed mp., IR in KBr, and VPC comparisons proving that dustanin is a x,22-dihydroxyhopane*.

The position of the secondary hydroxy group at C_{15} was established as follows. Oxidation of deoxydustaninone (V) with selenium dioxide in acetic acid afforded a dioxphenol (VIII), mp. 197-198°, $C_{30}H_{48}O_2$; λ_{max} 282mμ; IR 3413 (OH), 1678 and 1631cm^{-1} (CO-C(OH)=C), which showed no olefinic proton in the n.m.r. spectrum. Hence the possibility at ring A is ruled out. When dustaninone (III) was heated in 3% alcoholic hydrogen chloride for 1/2 hr., it produced an anhydro-ketone (IX) along with a small amount of a mixture of conjugated ketones (see below). The anhydro-ketone (IX), mp. 231-233°, $C_{30}H_{48}O$; IR 1684cm^{-1} (CO), gave a positive test to TNM but the double bond introduced was not conjugated with the ketonic function. Its n.m.r. spectrum showed no olefinic proton. Further acid treatment of IX, i.e. boiling for 2 hrs. in 5% alcoholic hydrogen chloride or on standing with 15% sulphuric acid in acetic acid for 12 hrs. at room temperature, isomerized it into a mixture of two conjugated ketones (Xa and Xb); one of which, iso-anhydrodustaninone-A was obtained in pure and had mp. 267-269°, $C_{30}H_{48}O$; λ_{max} 237mμ (ε=12700); IR 1647cm^{-1} (CO). The same mixture of the conjugated ketones was obtained directly from III on treatment with 6% sulphuric acid in acetic acid at 20° for 20 hrs. In n.m.r. spectrum

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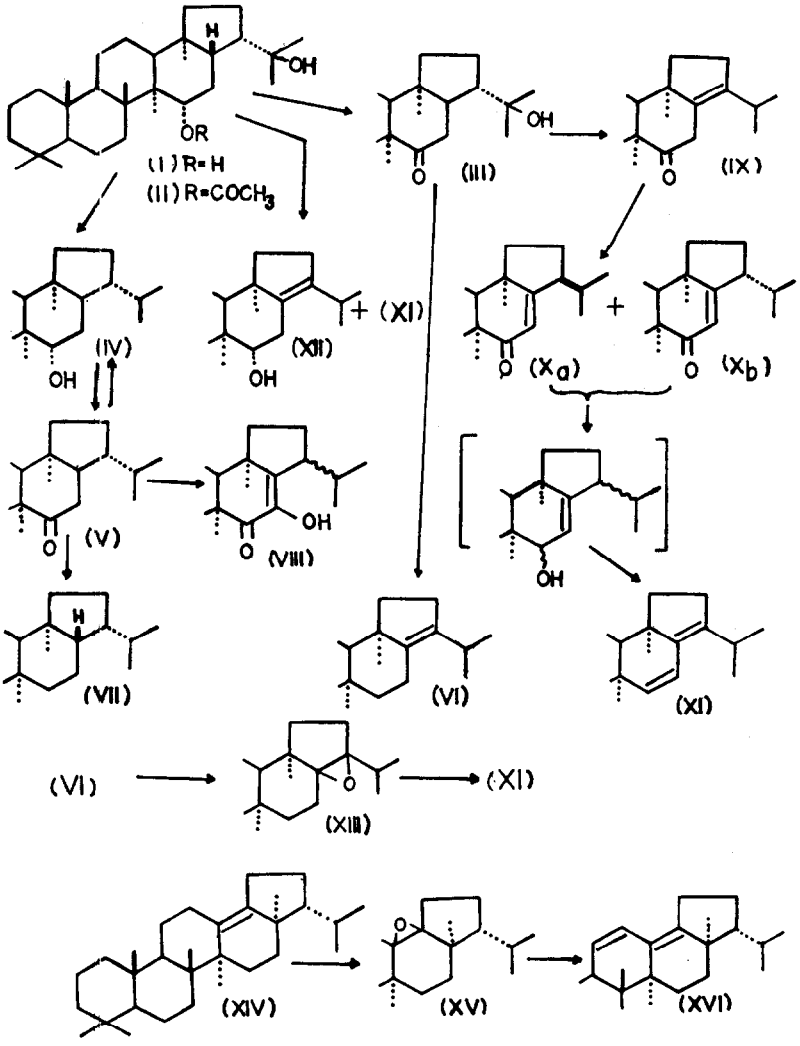
* Though there is an ambiguity on the identity of hopane and zeorinane since each saturated hydrocarbon derived from hydroxyhopanone and zeorin gave different peak in VPC (unpublished observation by the authors), we tentatively follow in this communication the proposed stereochemistries of zeorinane and zeorin (S. Huneck, Chem. Ber., 94, 614 (1961); S. Huneck and J. M. Lehn, Bull. Soc. Chim. France, 1702 (1963)) for representing those of dustanane and dustanin.


(I) R=H
(II) R=COCH3
(III)
(IX)
(IV)
(XII)
(XI)
(Xa)
(Xb)
(V)
(VIII)
(VII)
(VI)
(XI)
(XIII)
(XIV)
(XV)
(XVI)

isoanhydrodustaninone-A had one olefinic proton at 4.10τ(J=2c/s). The mixture of the conjugated ketones was then reduced with lithium aluminium hydride and the crude product was warmed with 3% alcoholic hydrogen chloride. The reaction afforded a diene, mp. 151-153°, λ_{max} 245, 252, and 261mμ, which was proved to be identical with hopa-15,17(21)-diene (XI) (see below). Hence isoanhydrodustaninone-A must be represented by Xa for which the configuration of the isopropyl group was assigned to be β from the considerable large allylic coupling constant of the olefinic proton; the situation resulted from the case in which the angle between C_{16}-H and C_{21}-H being close to 90°. The other conjugated ketone may be a stereoisomer (Xb), since it had very close mobility in TLC and the same UV absorption, although it was not obtained in pure state.

The synthesis of hopa-15,17(21)-diene (XI) was achieved as follows. Hopene-I (VI) was converted by monoperphthalic acid into an epoxide (XIII), mp. 272-273°, $C_{30}H_{50}O$. Acid treatment of XIII rapidly gave the diene (XI), mp. 153-155°, $[\alpha]_D$+61°, $C_{30}H_{48}$; λ_{max} 245, 252, and 261mμ (ε=18000, 21000, and 15000); N.m.r., AB quartet of 2H at 4.12τ (δ_{AB}=36c/s, J=10c/s). Identity of this with the diene obtained from dustanin was confirmed by mixed mp., UV, VPC, and TLC on silica gel-$AgNO_3$ comparisons. Similar treatment of hopene-II[2] (XIV) afforded hopene-II oxide (XV), mp. 202-204°, $C_{30}H_{50}O$ and then neohopa-11,13(18)-diene (XVI), mp. 213-215°, $C_{30}H_{48}$; λ_{max} 247, 256, and 267mμ(ε=23500, 28000, and 17700); N.m.r. 3.82 (1H, q, J_1=10c/s, J_2=3.5c/s), and 4.48τ (1H, d, J=10c/s). Its VPC peak was well separable from that of XI.

The diene (XI) was also obtained in a low yield by direct dehydration of dustanin (I) with 3% alcoholic hydrogen chloride along

with a major product, an anhydro-alcohol (XII), mp. 203-205°, $[\alpha]_D$+16°, $C_{30}H_{50}O$; IR 3571cm^{-1} (OH). The latter showed no olefinic proton in the n.m.r. spectrum.

Deoxydustaninone (V) when reduced by sodium in n-propanol quantitatively regenerated deoxydustanin (IV). Hence the hydroxyl group must be equatorial and α-oriented. Lithium aluminium hydride reduction of V gave the same alcohol (IV) as a major product, and the presence of an epimeric axial isomer of hydroxyl group in the product was shown by TLC as expected.

Dustanin thus proved to be hopan-15α,22-diol provides the first example of pentacyclic triterpenoid as a fungal metabolite.

There are two proporsals[3] for the occurence of triterpenoids lacking oxygenated function at C_3; i.e. they are formed from a corresponding C_3-oxygenated derivative by reduction or directly from squalene by a cyclization process initiated by H^+. Since all pentacyclic triterpenoids of non-rearranged type hitherto isolated from lichens[4] and micro-organisms[5] lack oxygenated function at C_3, we favor the latter possibility and suggest that in these livings of low division the different cyclization process from that in higher plants may be favorable; for the latters oxidative cyclization is more common.

The details of separation of dustanin from the fungus will be reported later by Dr. Yamamoto

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