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ISOLATION OF 3β-HYDROXY-4β-HYDROXYMETHYLFUSIDA-17(20)[16,21-cis],24-DIENE[*1]

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Recently the intact incorporation of the squalene chain into the fusidane skeleton was demonstrated and 3β-hydroxy-4β-methylfusida-17(20)[16,21-cis],24-diene (II) was proposed as the precursor of fusidic acid (III) (1). In this communication we wish to report the isolation of one of the precursors of helvolic acid (IV) whose structure is assigned to be 3β-hydroxy-4β-hydroxymethylfusida-17(20)[16,20-cis], 24-diene (V).

After a great part of IV of the metabolites mixture extracted from the mycelia of Cephalosporium caerulens was removed by recrystallization, the components of the mother liquor were chromatographed on silica gel column. Recrystallization of the eluate between ergosterol and IV afforded a diol[*2] (V), m.p. 143°, $C_{30}H_{50}O_2$[*3] M^+ 442, $[\alpha]_D^{20}$+19.1°, IR (CCl_4, C=0.0026 M. the calibration standard: indene) 3628±1 (sharp, nonbonded OH), 3567±1 cm^{-1} (broad, bonded OH), diacetate (VI), m.p. 96°, $C_{34}H_{54}O_4$, M^+ 526, $[\alpha]_D^{20}$+32.6°.

As shown in TABLE I, the NMR spectra indicate the existence of three methyls on the double bond, four tertiary methyls, and the partial structures >C<u>H</u>-OR and -C(-)-C<u>H</u>$_2$OR (R=H or Ac).

These NMR spectral data, the molecular formula, and the origin of this diol strongly suggested that this must be one of the precursors of IV in which a secondary and a primary hydroxy groups are most probably located at C_3-position

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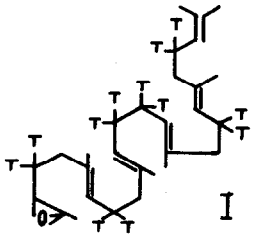
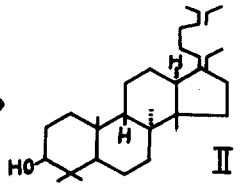
*1) This paper constitutes part V in the series on HELVOLIC ACID AND RELATED COMPOUNDS.
*2) This diol was kindly identified with the sample isolated from the culture of Fusidium coccineum (a private communication from Dr. W. O. Godtfredsen).
*3) The compound whose molecular formula is cited gave satisfactory analytical data. Unless otherwise stated, NMR (δ) and $[\alpha]_D$ were taken in $CDCl_3$ and $CHCl_3$ respectively.



I



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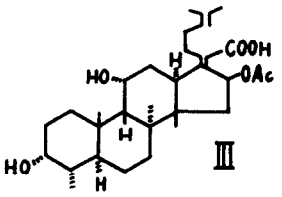

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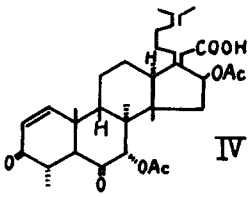
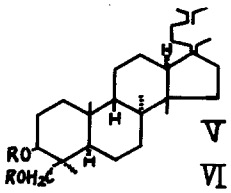


IV



V R : H
VI R : Ac

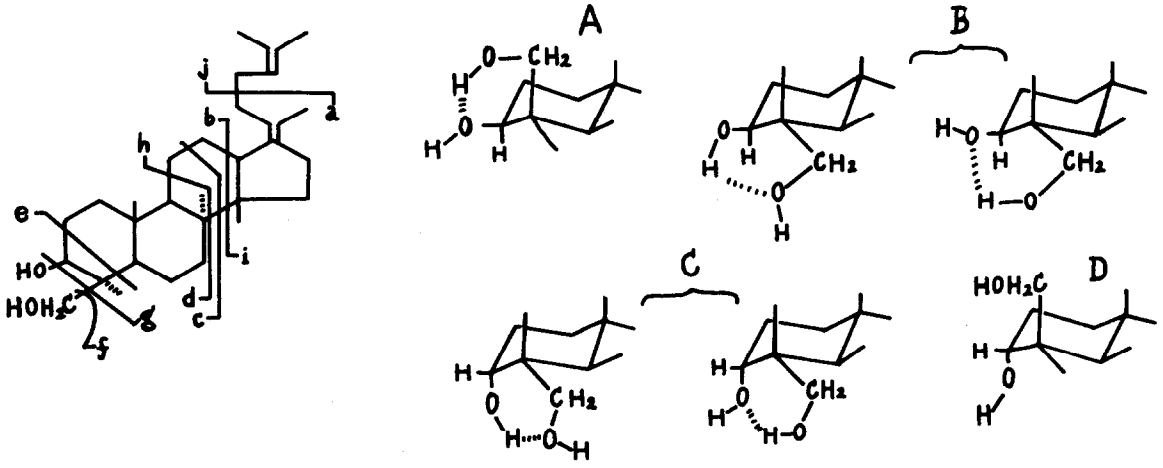
TABLE I

	$-\overset{OR}{\underset{|}{C}}-H$	$\underline{H}>C=C<$	$-\overset{|}{C}-\underline{CH_2}OR$	$>C=C<_{\underline{CH_3}}$	$-\overset{|}{C}-\underline{CH_3}$	$-O-\overset{O}{\overset{\|}{C}}-\underline{CH_3}$
V(R=H)	3.43[a] diffused t. J=ca 8	5.10 (m)	3.26 slightly diffused d, J=11.0[b] 4.21 d, J=11.0[b]	1.57 1.58 1.67	0.73 0.88 1.11 1.20	
VI(R=Ac)	4.60 diffused t. J=ca 8	5.10 (m)	4.16 d, J=12.0 4.29 d, J=12.0	1.59 1.60 1.67	0.76 0.97 1.02 1.14	2.03 2.05

a) The values obtained under addition of D_2O.
b) These signals were analyzed by the measurement under addition of D_2O utilizing spin-spin decoupling technique.

and at one of the two C_4-methyls. To confirm this assumption, 2.0mg of the diol ^3H-labeled by the Wilzbach method (2) (2.61×10^7 dpm/mg) was fed into a culture of C. caerulens (100 ml), preincubated for 2 days and continued cultivation for further 5 days. The usual work up followed by silica gel column chromatography and one recrystallization furnished IV, m.p. 214-5°, 14.45mg. After dilution with 73.20mg of cold IV, seven recrystallizations gave the specific activity 1.03×10^4 dpm/mg. This incorporation (1.71%) demonstrated that this compound is one of the intermediates in the main biogenetic path of IV and consequently the structure V can be assigned to it except the stereochemistry of C_3-OH and C_4-CH_2OH, which was elucidated as described below.

This proposed structure can rationalize the mass spectral data of this diol. The high resolution mass spectrum showed the peaks [$C_nH_mO_2$: 442(M), 373(a), 250(b-2), 237(c-1), 223(d-1), 101(e-1). $C_nH_mO_1$: 411(f), 355(a-H_2O). C_nH_m: 394(g), 218(h), 189(i-1), 69(j)].



The orientation of 3β-OH and 4β-CH_2OH could be assigned by the IR- and NMR-spectral studies. The IR-spectrum of the diol in the diluted CCl_4 solution exhibited only the absorption due to a nonbonded secondary OH of equatorial type at 3628 cm^{-1} but a nonbonded axial secondary (3637-3639 cm^{-1}) or a nonbonded primary OH (3640-3642 cm^{-1}) could not be observed. This fact cleary demonstrates that this compound possesses the partial structure (A: 3β-OH, 4β-CH_2OH, the preferred form of the axial primary OH away from the axial 10-methyl group), since the absorption due to a nonbonded primary OH should exist in all the other cases, (B: 3β-OH, 4β-CH_2OH, the equilibrium mixture of two forms), (C: 3α-OH, 4α-CH_2OH, the equilibrium mixture of two forms) and (D: 3α-OH, 4β-CH_2OH, no hydrogen bonding) (3).

In the NMR spectrum of diacetate the shape of the signal due to C_3-H (a slightly diffused triplet, J=ca 8 cps) is in accordance with that of the 3α-H (axial type, t., J=8, W/2=17) of the similar compound such as isoaescigenin pentacetate epoxide (4). On the other hand the average value ($\frac{Ha + Hb}{2}$=4.42) of the chemical shifts of C_4-CH_aH_bOAc of the diacetate is very similar to that (4.08-4.30), reported in the case of C_4-CH_2OAc of axial type but different from that (ca 3.84) of the equatorial type (5). Thus the stereochemistry of 3β-OH and 4β-

CH_2OH in the diol was also proved from the NMR-spectral data.

Consequently this diol, expected to be the first oxidation product of II, is assigned as 3β-hydroxy-4β-hydroxymethylfusida-17(20)[16,21-cis],24-diene (V).

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