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ISOLATION OF 3β-HYDROXY-4β-METHYLFUSIDA-17(20)[16, 21-cis], 24-DIENE (3β-HYDROXY-PROTOSTA-17(20)[16, 21-cis], 24-DIENE) AND A RELATED TRITERPENE ALCOHOL*1

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Recently 3β-hydroxy-4β-methylfusida-17(20)[16, 21-cis], 24-diene (I) has been assumed to be the first cyclization product of 2, 3-epoxysqualene in the biogenetic paths of fusidic acid (II), cephalosporin P_1 (III) and helvolic acid (IV) which possess the unique skeleton of fusidane type (1). This communication deals with the isolation and structural elucidation of this compound I and a related triterpene alcohol VIII.

After filtering the precipitated helvolic acid from the concentrated methanol solution of the metabolite mixture extracted from Cephalosporium caerulens (3000 L of culture broth containing mycelia), the components of mother liquor were subjected to a silica gel column chromatography. Two triterpene alcohols, I (546 mg.) and VIII (882 mg.), were obtained; I, $C_{30}O_5O$*2, m.p. 104-105°, $[\alpha]_D^{CHCl_3}$: +10° ±2°, VIII, $C_{30}O_5O$, m.p. 117-117.5°, $[\alpha]_D^{CHCl_3}$: +16°±2°.

The molecular formula and the spectral data of the former alcohol could account for the structure I. To confirm this structure, a chemical correlation between I and the known triterpene diol V (2) was attempted. Partial tosylation (3) of V afforded a monotosylate (VI) as a main product, $C_{37}H_{56}O_4S$, m.p. 149-149.5°, whose NMR spectrum showed the peaks (4.08, J=ca 8.0, 4.16, J=ca 8.0 cps) due to $-CH_2-OTs$. A treatment of VI with excess amount of $LiAlH_4$ in tetrahydrofuran at

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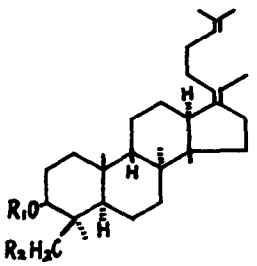
*1) This paper constitutes part VI in the series on HELVOLIC ACID AND RELATED COMPOUNDS.

*2) The compounds whose molecular formula are cited gave satisfactory analytical data.

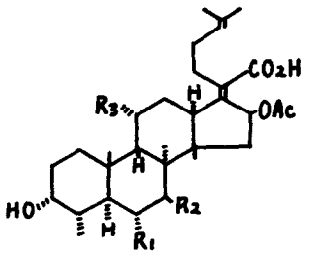
TABLE I

compound (solvent)	$-\overset{OH}{\underset{|}{C}}-H$	$\overset{H}{\diagdown}C=C\diagup$	$\diagdown C=C\diagup CH_3$	$-\overset{H}{\underset{|}{C}}-CH_3$	$-\underset{|}{\overset{|}{C}}-CH_3$
VIII (CCl_4)	3.10 m	4.98 m a)	1.51 s a) 1.62 s a)	0.95 d. J=ca 6	0.75 0.92 (6H) 0.95 (6H) 1.05
I (CCl_4)	3.13 m	5.02 m	1.56 1.56	—	0.76 (6H) 0.93 0.95 1.13
V ($CDCl_3$)	3.43 diffused t. J=ca 8	5.10 m	1.57 1.58 1.67	—	0.73 0.88 1.11 1.20
Lanosterol ($CDCl_3$)	3.18 m	5.05 m	1.58 1.65	0.85 d. J=ca 6	0.69 0.80 0.86 (6H) 0.98 (6H)

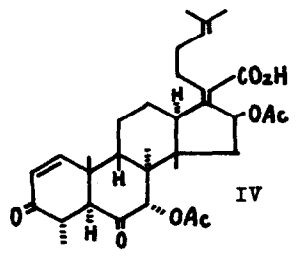
a) The spin-spin decoupling demonstrated the partial structure $\overset{CH_3}{\underset{CH_3}{\diagdown}}C=CH-$.



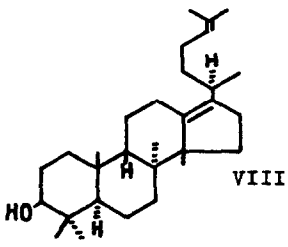
I: $R_1=R_2=H$
V: $R_1=H$, $R_2=OH$
VI: $R_1=H$, $R_2=OTs$
VII: $R_1=Bz$, $R_2=H$



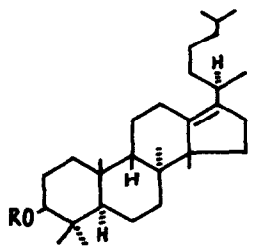
II: $R_1=R_2=H$, $R_3=OH$
III: $R_1=OAc$, $R_2=OH$, $R_3=H$



IV



VIII



IXa: R=H
IXb: R=Ac
IXc: R=Bz

room temperature and subsequent benzoylation afforded a monobenzoate (VII), $C_{37}H_{54}O_2$, m.p. 158-159.5°, identical with the monobenzoate of I in all respects, i.e., IR-, NMR-spectra, $[\alpha]_D^{CHCl_3}$: VII=+44±4°, monobenzoate of I=+42±2°, and the mixed melting point test. Thus the structure of I was determined to be 3β-hydroxy-4β-methylfusida-17(20)[16, 21-cis], 24-diene.

The combinatory spectroscopy of the other alcohol VIII is suggestive of a structure closely related to that of I. Catalytic hydrogenation of VIII over Pd-C in ethanol gave a dihydro derivative IXa, $C_{39}H_{52}O$, m.p. 115° (MeOH). Its acetate IXb, $C_{32}H_{54}O_4$, m.p. 83-84° (MeOH), ORD (THF): [α]=+41.4° (350 mμ), +37.6° (400 mμ), +24.8° (500 mμ) and +20.3° (600 mμ), and its benzoate IXc, $C_{37}H_{56}O_2$, m.p. 123-124° (EtOH), $[\alpha]_D^{CHCl_3}$=+44.1°, NMR($CDCl_3$): 0.8-1.14 (three secondary methyls and five tertiary methyls), were obtained as monoclinic crystals by crystalizations from very dilute solutions. On the other hand a cetalytic hydrogenation of I over Pd-C in hexane resulted in the formation of a mixture mostly composed of a dihydro and a tetrahydro derivatives with a ratio of ca 4 : 1. The acetate, m.p. 82-84°, ORD (THF): [α]=+41.3° (350 mμ), +37.4° (400 mμ), +25.3° (500 mμ) and +20.0° (600 mμ), and the benzoate, m.p. 122-124°, $[\alpha]_D^{CHCl_3}$=+44.2°, of this dihydro derivative were identical with the acetate IXb and the benzoate IXc derived from VIII, respectively, in all the spectral data and in the mixed melting point tests. The identity of the two benzoates was further supported by taking the X-ray diffraction photographs of b-axis oscillation, whole rotation and zero layer Weissenberg. The lattice constants and the intensity distributions of the two compounds proved to be the same. The spectral data of IXb and IXc demonstrated that their tetrasubstituted double bonds are located not at C_{17}-C_{20} but, instead, at C_{13}-C_{17} position, which requires an isomerization of C_{17}-C_{20} double bond of I in the course of the catalytic hydrogenation. The identity of the dihydro derivatives from both I and VIII indicates that compound VIII has the same structure as I except the position of the tetrasubstituted double bond. Consequently the structure of VIII was determined except the configuration at C_{20}. This compound was also identified with a triterpene alcohol isolated from the metabolite mixture of Fusidium Coccineum, whose structure was recently elucidated to be VIII by chemical correlation with lanosterol*3 and, therefore, the configuration at C_{20} was also determined to be R.

It is of interest to note that this double bond isomerization on catalytic hydrogenation of I yielded only one configuration at C_{20} which is assumed to be the

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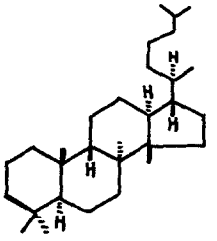
*3) Dr. Godtfredsen kindly carried out the comparison of these two specimens. The structure VIII was assigned for this triterpene alcohol by an interrelation with lanosterol. (Private communication from Dr. Godtfredsen and Professor Arigoni).

same as that of VIII. This result will be discussed elsewhere.

Scince these hydrocarbons I and VIII are considered to be proto type derivatives of sterols, we propose the structure X as ''protostane''*4 and, accordingly, compounds I and VIII will be called 3β-hydroxy-protosta-17(20), (16, 21-cis], 24-diene and 3β-hydroxy-protosta-13(17), 24-diene, respectively.



X

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   b) F. G. Gault, J. J. Rooney, C. Kemball, J. Catalysis, 1, 255 (1962).

*4) This name for X was suggested to us by Dr. Godtfredsen as a result of the discussion with Professor Arigoni.