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FERN CONSTITUENT; COLYSANOXIDE, AN ONOCEROID HAVING A NOVEL CARBON SKELETON, ISOLATED FROM <u>COLYSIS</u> SPECIES

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Abstract: A new triterpenoid named colysanoxide (II) having a novel carbon skeleton has been isolated from the leaves of *Colysis elliptica* and *C. pothifolia*, together with α-onoceradiene from the latter. The structure of II was established by spectroscopic, chemical and X-ray diffraction methods.

In a previous paper[1)] the three onoceroid triterpenes, α-onoceradiene (I), serratene and onoceranoxide, were reported from the Polypodiaceous ferns, *Lemmaphyllum microphyllum* PR. varieties. In connection with triterpenoids of the same family, a new compound, named colysanoxide (II) was isolated from the dried leaves of *Colysis elliptica* CHING ("Iwa-hitode" in Japanese, yield 0.021%) and *C. pothifolia* PR. ("Oo-iwa-hitode, 0.027%), together with I from the latter fern. This paper deals with the structure establishment of compound II by spectroscopic and chemical methods as well as by a X-ray diffraction method.

Colysanoxide (II), mp. 199-201.5°C, $[\alpha]_D^{23}$ -59.6° ($CHCl_3$, c=0.7), Rt_R 2.22,[2)] was shown to have the molecular formula $C_{30}H_{52}O$ by MS (M^+ m/z 428.4019, 0.3% of the base peak; M^+-CH_3 413.3781, 4) and ^{13}C-NMR spectra (30 carbon signals were observed). As the absence of a hydroxyl, a carbonyl groups (IR spectrum) or a double bond (tetranitromethane test and ^{13}C-NMR spectrum) was shown, compound II should be pentacyclic including one epoxy ring. The ^{13}C-NMR spectrum of II (TABLE) exhibited six singlets including two carbon signals at δ74.0 and δ75.8 to be assigned for the carbons bearing

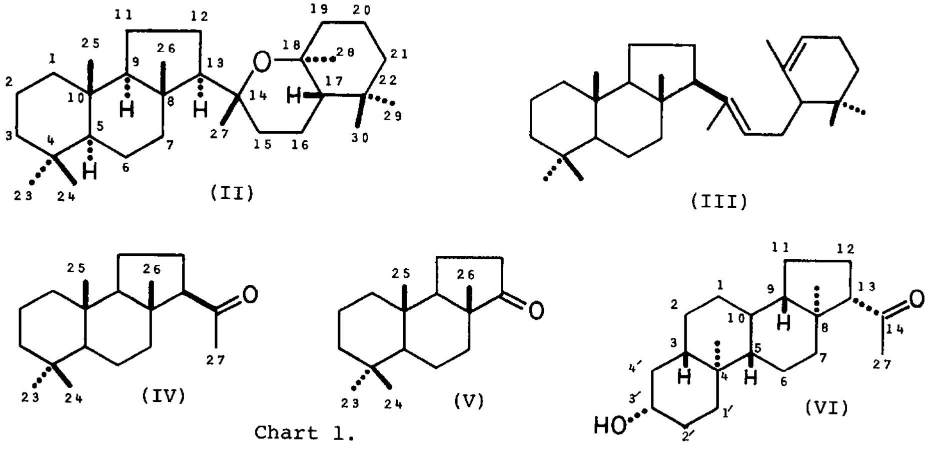

Chart 1.

a methyl group and a oxygen atom, four doublets, twelve triplets and eight quartets (OFR), while the ^{1}H-NMR spectrum of II gave eight tertiary methyl signals (TABLE), and methylene and methine signals only.

To clarify the carbon skeleton, compound II was treated with 20% BF_3-etherate in ether solution at 20°C to give a mixture of dienes, the main product of which was later concluded to be III by GC-MS (Rt_R 1.62, M^+ m/z 410.4537, $C_{30}H_{50}$, the other flagments are shown in Chart 2) and ^{1}H-NMR spectrum (TABLE). The products without complete purification (the products were rather unstable) was subjected to ozonolysis followed by zinc powder and acetic acid treatment to afford a methyl ketone (IV),3) mp. 68-70°C, $[\alpha]_D^{23}$ -123.5° (CHCl , c=0.7), $C_{19}H_{32}O$ (MS, M^+ 276.2441), IR $\nu_{cm^{-1}}^{KBr}$ 1709, together with a small amount of a five membered ring ketone (V), $C_{17}H_{28}O$ (MS, M^+ 248.2129), IR $\nu_{cm^{-1}}^{KBr}$ 1742. The structure of IV was confirmed by the MS fragments (Chart 2), and ^{1}H- and ^{13}C-NMR spectra (TABLE) comparing with those of pregnanolone (VI) prepared from pregnenolone available commercially. The compound V also indicated the reasonable data in its MS fragments (Chart 2) and ^{1}H-NMR spectrum (TABLE). As the solvent shifts values ($\delta_{C_6H_6}$-δ_{CHCl_3}) of the methyl signals were highly negative both of the compounds IV [(C24) -0.022, (C25) -0.135, (C26) -0.049] and VI [(C24)-0.171, (C26) -0.034] , the methyl ketone side chains of IV and VI should be fixed to the same direction like the presence of a hydrogen bonding between the methyl (C26) proton and the carbonyl oxygen. Hence comparison of the ORD and CD curves of IV with those of VI should be quite effective to establish the absolute configuration of IV. The fact that the ORD and CD curves of IV ($[\Phi]_{312}$ -4890°, $[\Phi]_{269}$ +6940°; $[\Theta]_{294}$ -12600°) and those of VI ($[\Phi]_{313}$ +5960°, $[\Phi]_{267}$ -7430°; $[\Theta]_{294}$ -12060°) were opposite sign, established the absolute configuration of IV to be shown in Chart 1 comparing with that of VI.

TABLE. Chemical Shifts (δ) for $CDCl_3$ Solution (JEOL JNM-FX 100)

	Proton(s) attached to C( )a)c)								
	23	24	25	26	27	28	29	30	others
II	0.840	0.811	0.840	0.899	1.257	1.186	0.801	0.840	
III	0.877	0.823	0.845	0.620	1.588	1.563	0.845	0.877	(15)5.159*bt*(6.6) (19)5.256*m*
IV	0.867	0.830	0.855	0.703	2.081				
V	0.865	0.850	0.982	0.948					
VI		0.811		0.605	2.108				

	^{13}C of C( )b)c)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
II	40.5	18.5	42.7	33.0	57.6	19.3	43.3	44.5	63.6	37.3	19.6	23.2	62.7	74.0	31.5
IV	40.3	18.4	42.6	33.0	57.5	19.5	41.4	44.7	63.5	37.3	19.8	22.4	65.2	209.4	
VI	32.0	28.6	44.8	35.5	54.3	21.3	38.1	44.2	56.7	35.5	24.4	22.8	63.8	209.5	

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
II	15.5	47.9	75.8	43.3	20.5	42.2	33.9	33.5	21.3	15.6	16.0	28.5	24.8	20.5	32.0
IV								33.5	21.3	15.5	15.9	31.3			
VI									12.3		13.4	31.4			

a) Assignments were confirmed by $CDCl_3$-C_6D_6 solvent shifts (II,IV,V,VI) and Lanthanide shifts (II,IV,VI). b) Assignments were made by comparison with those of cholesterol,4) *ent*-beyer-16-ene,5) hopane,6) dihydromanoyl oxide7) and their related compounds, and were confirmed by $CDCl_3$-C_6D_6 solvent shifts and Lanthanide shifts on PRFT method.
c) Numbering of the carbons of IV,V and VI were made conveniently to correspond with those of I as shown in Chart 1. The other chemical shifts of VI were (1') 37.1, (2') 31.4, (3') 71.1 and (4') 38.1.

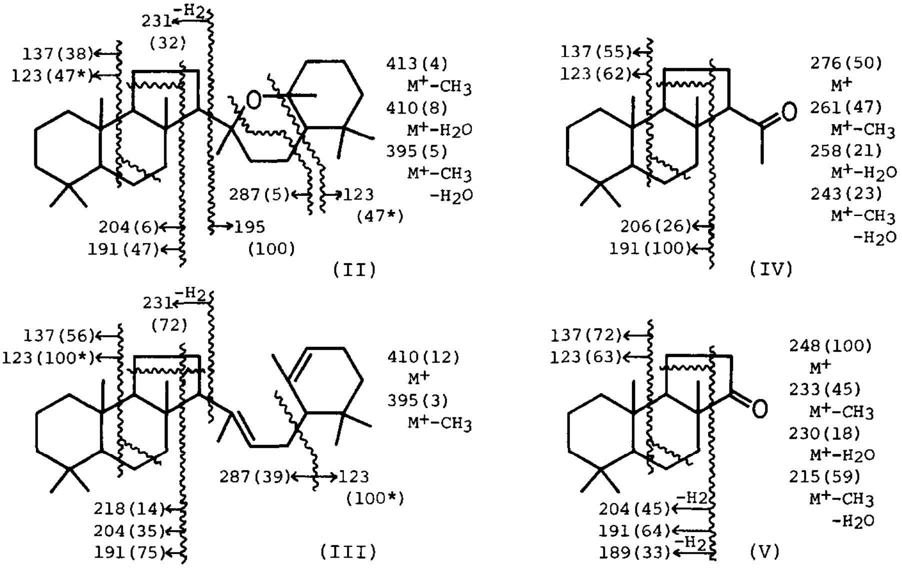

Chart 2. MS Fragments of II - V (JEOL D-300)

Although any other products of the above-mentioned degradation reaction were not identified unfortunately, it can be concluded that compound II has a tricyclic ring system with four methyl groups like V, and also one methyl group and a epoxy oxygen attached to the first carbon (C14) on the side chain. The remaining part of II except the tricyclic ring system could be bicyclic and was caluculated to be $C_{13}H_{23}O$, which was exactly coincided with the base peak, m/z 195.1765, in the MS spectrum of II. Thus the gross structure of II except stereochemistry at C14 can be formulated as shown in Chart 1 to satisfy ^{1}H- and ^{13}C-NMR (TABLE), and MS spectra (Chart 2), as well as biogenetical and chemotaxonomical points of view (colysanoxide was supposed to be a onoceroid triterpene).

In order to define unambigously the structure and stereochemistry of II, Osaka group of the authors tried to determine its crystal structure by X-ray diffraction method. A crystal of II grown from acetone-ether, size 0.2X0.5X0.3 mm, was mounted on a Rigaku automatic four-circle diffractometer equipped with a graphite monochrometer. *Crystal data*: $C_{30}H_{52}O$, MW 428.7, monoclinic, a=11.792(3), b=7.163(1), c= 16.457(4) Å, β=109.69(2)°, V=1308.8(5) Å^3, ρ_o=1.085(1) g·cm^{-1}(H_2O- KI saturated sol.), ρ_c=1.088 g·cm^{-1}, Z=2, space group $P2_1$. Reflections were scanned up to Θ=65° using Cu-Kα radiation. Of the 2484 reflections measured, 2293 having $I \geq 2\sigma(I)$ were used for the following structure determination and refinements. The structure was solved by the direct method (MULTAN program[8]), and refined by the block-diagonal least-squares method with anisotripic temperature factors for nonhydrogen atoms and isotropic ones for hydrogen atoms to R factor of 0.072. An ORTEP drawing of the molecular structure of II is shown in Fig.[9]

As far as the conformation of the crystalline II concern, the pyran ring is a boat form being C18→C28 methyl group a flagpole, and the two ring systems are fixed

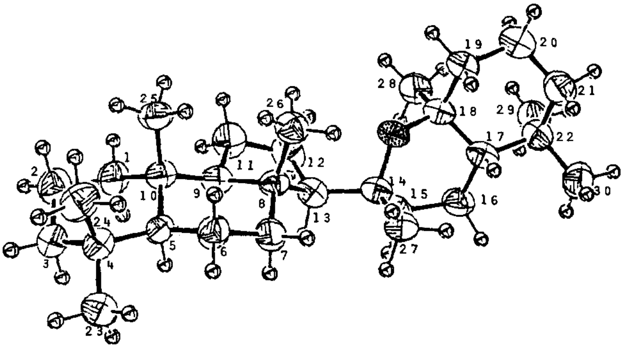

Fig. An ORTEP Drawing of the Molecular Structure of Colysanoxide (II)

like C14→oxygen and C8→C26 methyl group being almost parallel. The latter fact could also be the same in a $CDCl_3$ solution, because the chemical shift of C26 methyl group of II was observed at a remarkably lower field as shown in the TABLE.

Colysanoxide is the first example of a vegetable triterpenoid having an unsymmetrical onoceroid skeleton,[1] to which the name colysane will be applied for the saturated hydrocarbon. The compound is also very interesting from a chemotaxonomical point of view, because other *Colysis* species in Japan, such as *C.* ×*shintenensis* H. ITO ("Shinten-uraboshi"), *C.* ×*simplicifrons* TAGAWA ("Hitotsuba-iwahitode") and *C. wrightii* CHING ("Yarinoho-kuriharan"), indicated the presence of the compound by GC-MS, while other Polypodiaceous ferns being studied did not at all.

References and Footnotes

1) A. Ageta, K. Shiojima, K. Masuda, *Chem.Pharm.Bull.*, 30, 2272 (1982).

2) GLC were run by Hitachi 163 on Chromosorb G HP coated with SE-30 (1.4%) at 260°C in a flow of nitrogen. Cholestane was used as a reference and its retention time was set at 3.5 min. Incidently, Rt_R of II was just the same with that of onoceranoxide, but MS spectra were effective to distinguish II from the latter.

3) Compound IV and the degradation product of malabaricol (R.R. Sobti, Sukh Dev, *Tetrahedron Lett.*, 1968, 2215; W.F. Paton, I.C. Paul, A.G. Bajaj, Sukh Dev, *Ibid.* 1979, 4153) were proved to be entirely different at the configuration of C13 by comparison of both samples (^{1}H-NMR, ORD, CD and MS). Thanks are due to Dr. Sukh Dev for sending the sample.

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9) Final crystallographic coordinates and a table of bond distances have been deposited with the Cambridge Crystallographic Data Centre.

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