STRUCTURE OF HERBARIN

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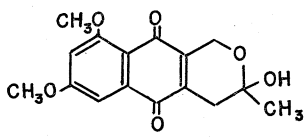
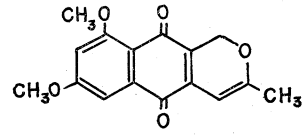
Herbarin and dehydroherbarin, isolated from the fungus *Torula herbarum* (PERS.), belong to the class of pigments in which a naphthaquinone moiety is fused to an oxacyclohexene ring at the 1, 2-positions. Their structures are discussed.

Herbarin (I) and dehydroherbarin (II) isolated from the fungus *Torula herbarum* (PERS.)[1] possess weak antimicrobial activity and belong to the class of pigments in which a naphthaquinone moiety is fused to an oxacyclohexene ring at the 1, 2-positions. The first compounds characterized in this class were javanicin (III) and fusarubin (also called oxyjavanicin) (IV) from the fungus *Fusarium javanicum*[2,3,4]. Javanicin does not possess a tricyclic structure, but its biogenetic relationship to the tricyclic congener, fusarubin, is evident. Subsequently, SCHMID and co-workers isolated



I



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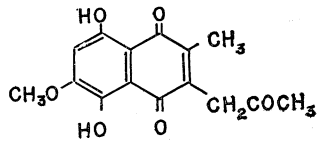

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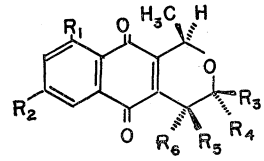
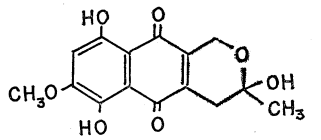


V $R_1 = OCH_3$; $R_3 = CH_3$; $R_2, R_4, R_5, R_6 = H$

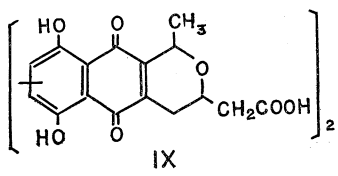
VI $R_1 = OCH_3$; $R_4 = CH_3$; $R_2, R_3, R_5, R_6 = H$

VII $R_1, R_2 = OCH_3$; $R_4 = CH_3$; $R_5 = OH$; $R_3, R_6 = H$

VIII $R_1, R_2 = OCH_3$, $R_4 = CH_3$, $R_6 = OH$ $R_3, R_5 = H$



IV



IX

eleutherin (**V**) and isoeleutherin (**VI**) from the tubers of *Eleutherine bulbosa*[5,6,7]. TODD and his group proved that the naphthaquinones **VII** and **VIII** obtained by reduction of protophins, the coloring matter of *Aphididae*, belong to this class of compounds[8,9]. Actinorhodin (**IX**), produced by the mold *Streptomyces coelicolor*, has a dimeric naphthaquinone structure[10].

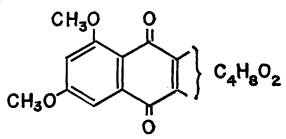
Herbarin is a neutral compound with composition $C_{16}H_{16}O_6$. Its absorption spectrum is similar to the spectra of 5, 7-dimethoxy-1, 4-naphthaquinone [λ_{max} 412, 258 and 216 nm (log ε 3.55, 4.17 and 4.53), $\lambda_{infl.}$ 360 nm (log ε 3.38)], and the naphthaquinone dimethyl ethers **VII** [λ_{max} 412, 267 and 216 nm (log ε 3.69, 4.36 and 4.66)] and **VIII** [λ_{max} 412, 268 and 216 nm (log ε 3.63, 4.25 and 4.59)][8]. The infrared spectrum of herbarin and that of **VII** and **VIII** are similar, showing the presence of bands due to hydroxyl and quinone groups. High resolution mass spectrum shows the molecular ion of herbarin at 304.0905; the calculated value for $C_{16}H_{16}O_6$ is 304.0947. Analysis of the high resolution mass spectrum shows that, in addition to the presence of the characteristic $M^{\oplus}$-CO peak due to quinones, there are peaks due to $M^{\oplus}$-H_2O and $M^{\oplus}$-CH_3, indicating the presence of hydroxyl and methyl groups.

Table 1. NMR spectral data of herbarin (I)[a]

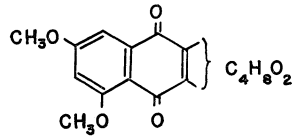
Solvent	H-5 and H-7	H-9e′	H-9a′	Two OCH_3	H-12e′	H-12a′	11-CH_3	11-OH[b]
DMSO-d_6	2.87, 3.04 J=2.3	5.50 (m)		6.04, 6.08(s)	7.4~7.7 (m)		8.55(s)	4.01(s)
Pyridine-d_5	2.62, 3.18 J=2.5	4.95 (m)		6.17, 6.21(s)	6.86 (m) J=18	7.33 (m) J=18	8.26(s)	5.4
DMF-d_7	2.84, 3.02 J=2.3	5.42 (m)		5.99, 6.01(s)	7.31 J=18	7.59 J=18	8.51(s)	6.0 (s)
$CDCl_3$: CD_3OD 3 : 1	2.75, 3.29 J=2.5	5.28, J= 19, 2.7, 1.0	5.65, J= 19, 4.0, 3.0	6.05, 6.06(s)	7.18, J= 19, 3.0, 1.0	7.54, J= 19, 4.0, 2.7	8.51(s)	8.45(s)

a) Chemical shifts are expressed in τ values, and coupling constants in Hz. (s)=singlet; (m)=multiplet.
b) This proton is exchangeable with D_2O.

Herbarin was not sufficiently soluble in chloroform, so its nmr spectrum was determined in other solvents (See Table 1). Though these spectra afforded some information about structure, they were not amenable to complete analysis. However, the nmr spectrum of herbarin in a mixture of deuterated chloroform and methanol yielded valuable information (See Table 1). The chemical shifts and coupling constants of the two doublets at 2.75 and 3.29 τ with J=2.5 Hz suggest that these are aromatic protons having a meta relationship. A double irradiation experiment confirmed that these two protons are coupled by 2.5 Hz. The chemical shifts of the three proton singlets at 6.05 and 6.06 τ suggest that they are due to aromatic methoxyl



Xa

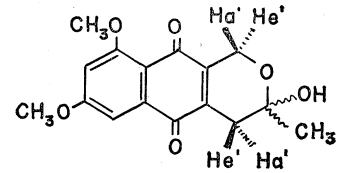


Xb

groups. On the basis of the data presented above, the partial structure of herbarin can be written as **Xa** or **Xb**.

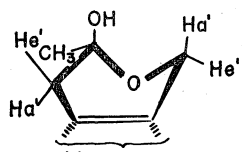
In the nmr spectrum there is a three-proton singlet at 8.51 τ. On dehydration of herbarin to dehydroherbarin, this singlet moves downfield to 8.02 τ characteristic of a methyl group on a double bond[1]. Further, in the DMSO-d_6 spectrum of herbarin there is one proton exchangeable singlet at 4.01 τ. These data suggest the presence of a tertiary hydroxyl[11] and a tertiary methyl on the same carbon atom. The two protons at 5.28 and 5.65 τ with coupling constants of 19 Hz are consistent with an AB-type system of a methylene group adjacent to an oxygen atom. These chemical shifts are in good agreement with the chemical shifts of H-9 protons of the naphthaquinones **V**~**VIII**[9].

The two protons at 7.18 and 7.54 τ with J=19 Hz suggest that they are due to the presence of benzylic AB-type methylene group. The chemical shifts are in agreement with the H-12 protons of the naphthaquinones **V**~**VIII**[9]. Consequently, the structure of herbarin can now be expanded to **Xc** (or the 6, 8-dimethoxy isomer of **Xc**).

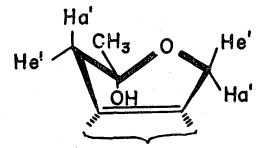


Xc

Double irradiation showed that all the other coupling constants observed in the C-9 and C-12 methylene protons originate from five bond homoallylic long-range coupling and from analogy to earlier work[9]; the long-range coupling constants in herbarin are $J_{9a'\,12a'}=4.0$, $J_{9a\,12e}=3.0$, $J_{9e'\,12a'}=2.7$, and $J_{9e'\,12e'}=1.0$ Hz. A comparison of the chemical shifts of herbarin in chloroform-d_3 and pyridine-d_5 shows that whereas H-12a′, H-12e′, H-9e′, and 11-CH_3 undergo a small chemical shift of 20~35 Hz, H-9a′ experiences a large 70 Hz downfield shift. Herbarin has a number of oxygen functions, and it is not certain that conclusions arrived at by a study of pyridine-induced solvent shifts in simple hydroxylic compounds is applicable here[12,13]. However, if this large downfield shift is assigned to the 1, 3-diaxial relationship of the hydroxyl and the H-9a′ proton, the conformational structures, **Xd** and **Xe**, are possible for the oxacyclohexene ring of herbarin. The conformation in **Xd** [(11 *R*)-



Xd 11*R*



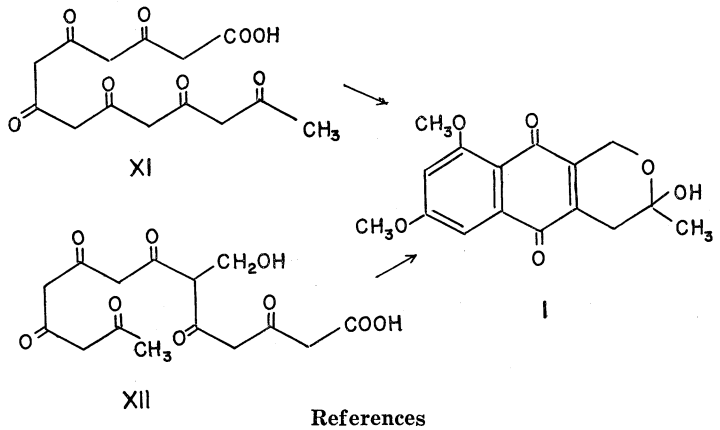
Xe 11*S*

herbarin] correspond to isoeleutherin, and that in **Xe** [11 *S*)-herbarin] to eleutherin, respectively[9]. From the present data, it is not possible to confidently assign the *R* or the *S* configuration to herbarin. The two methoxyls in herbarin could be at the 6- and 8-, or at 5- and 7-positions.

Two groups of workers have suggested that the acetate derived poly-β-keto acids, **XI** and **XII**, are the biogenetic precursors for fusarubin and javanicin[14,15,16], and were able to resolve the uncertainty of the location of the methoxyl group. It seems attractive to postulate that one of these poly-β-keto acids is also the biogenetic precursor

for herbarins and protoaphins[8]. This biogenetic argument suggests that the two methoxyls in herbarin are at 6- and 8-positions.

Herbarin could be converted to dehydroherbarin, and the physical-chemical data of dehydroherbarin[1] is in excellent agreement for structure II.



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