STRUCTURE OF ARCAPILLIN, AN ANTIHEPATOTOXIC PRINCIPLE OF ARTEMISIA CAPILLARIS HERBS[1]

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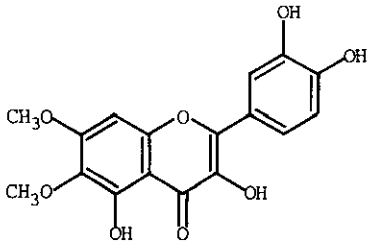
Abstract — In view of the observation that a methanol extract of Artemisia capillaris herbs has shown intense suppressive activity in carbon tetrachloride-induced liver lesion in mice, the extract has been fractionated by monitoring the hepatotoxic activity to furnish the known flavonol, eupatolitin (III), and a new flavone, arcapillin, as the liver-protective principles. The structure of arcapillin has been elucidated as that represented by formula I on the basis of chemical and physical data.

The crude drug "inchinkō", the aerial part of Artemisia capillaris Thunberg (Compositae), has been said to have choleretic and liver-protective effects and utilized as a remedy for hepatitis in Oriental medicine. From this crude drug, a number of terpenoids, acids, polyenynes, phenols, coumarins, chromones and flavonoids have been isolated. Among these constituents, capillarisin[2] and 6,7-dimethylesculetin[3] were reported to show choleretic activity. No liver-protective principles, however, have been reported.

During the course of our survey for liver-protective principles in crude drugs, we found that methanol extracts of this crude drug exhibited antihepatotoxic activity in carbon tetrachloride-induced liver lesion in mice although its potency varied remarkably depending on lots.[4] The extract was, therefore, fractionated by means of chromatography over polyamide and silica gel by monitoring this physiological activity to obtain, besides a flavonol, a novel flavone which has now been termed as arcapillin.

The flavonol, m.p. 280-283°, $C_{17}H_{14}O_8$ (MS: m/z 346 (M^+)) showed the following spectral properties:[5] UV λ_{max} nm (log ε): 211.5 (4.45), 259 (4.38) and 363 (4.28); IR ν_{max} cm^{-1}: 3200 (hydroxyl) and 1650 (enone); ^1H NMR δ: 3.66 (3H s), 3.95 (3H s), 6.86 (1H s), 6.91 (1H d, J 8), 7.61 (1H dd, J 8, 2), 7.76 (1H d, J 2) and 12.47 (1H s). These data were in good accord with those reported for eupatolitin (III).[6,7] The UV absorption spectra in the presence of aluminum chloride-hydrochloric acid, sodium acetate and sodium hydroxide indicated the location of hydroxyls at $C_{(3)}$ and $C_{(5)}$ and a methoxyl at $C_{(7)}$. The mass spectral peaks at m/z 137 and 109 arising out of B-ring demonstrated the presence of two hydroxyl groups in B-ring. Further, direct comparison of the UV and mass spectral data with those of eupatolitin (III) revealed that both sets of data were identical. Based on the above evidence, this flavonol was concluded to be eupatolitin (III).



III

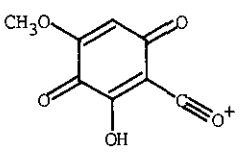
Arcapillin, m.p. 272-274°, $C_{18}H_{16}O_8$ (HRMS: m/z 360.0836 (M^+)) was shown to be a flavonoid in nature from its positive

response to ferric chloride, magnesium-hydrochloric acid and zinc-hydrochloric acid tests. In the IR spectrum, bands for hydroxyls (3460 cm^{-1}) and hydrogen-bonded carbonyl (1655 cm^{-1}) were observed. The UV spectrum disclosed an absorption maximum at 264 nm attributable to the benzoyl moiety in A-ring of a flavonoid (Band II) which showed red shift by 11 nm on addition of aluminum chloride.[8] Consistent with these findings, a signal at δ 13.08 due to chelated hydroxyl was found in the ^{1}H NMR spectrum and a signal at δ 183.5 assignable to carbonyl carbon was discernible in the ^{13}C NMR spectrum. These data indicated arcapillin to be a 5-hydroxyflavone.

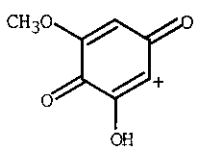
In the UV spectrum, an absorption maximum at 369 nm (Band I) showed longer wavelength shift by 70 nm but caused no essential alteration in intensity on addition of sodium hydroxide, demonstrating the presence of a hydroxyl group at $C_{(4')}$.[8] When aluminum chloride and hydrochloric acid were added, the absorption maximum at 369 nm (Band I) displayed displacement toward longer wavelength by 29.5 nm, a fact which revealed that an oxygen function such as hydroxyl or methoxyl was located at $C_{(6)}$ in 5-hydroxyflavone.[9]

The ^{1}H NMR spectrum exhibited, in addition to the signal at δ 13.08 for the $C_{(5)}$-hydroxyl, signals at δ 3.76, 3.84 and 3.96 attributed to three methoxyl groups. These facts together with its molecular composition indicated that arcapillin possessed three hydroxyls and three methoxyls. Further, in the ^{1}H NMR spectrum, there were signals at δ 6.60 for the $C_{(3)}$-hydrogen, δ 6.97 for the $C_{(8)}$-hydrogen, and δ 7.13 and 7.47 for two hydrogens in B-ring. The latter two signals appearing as singlets indicated the two hydrogens in B-ring to be in <u>para</u> position. Consequently, the hydroxyls and/or methoxyls were located at $C_{(2')}$, $C_{(4')}$ and $C_{(5')}$ in B-ring. Because an NOE (23%) was observed between the singal at δ 3.84 due to a methoxyl and that at δ 7.47 assignable to the $C_{(6')}$-hydrogen, the presence of the methoxyl at $C_{(5')}$ was deduced.

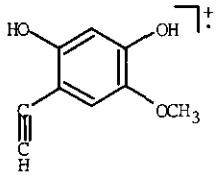
Since the mass spectrum of arcapillin showed, besides the molecular ion peak at <u>m/z</u> 360, an intense ion peak at <u>m/z</u> 345, the location of a methoxyl at $C_{(6)}$ was inferred.[10] Further, ion peaks at <u>m/z</u> 181 (A) and 153.0221 ($C_7H_5O_4^+$) (B) formed by retro Diels-Alder type fission of C-ring and an ion peak at <u>m/z</u> 164.0436 ($C_9H_8O_3^+$) (C) originating from B-ring were observed. The mass spectrum of the triethyl ether, prepared from arcapillin by treatment with ethyl iodide and potassium carbonate in dimethylformamide, gave an ion peak at <u>m/z</u> 181 (D) generated from A-ring which, concordant with introduction of the ethyl group, showed a shift by a C_2H_4 unit as compared with the corresponding ion peak (B) in arcapillin.



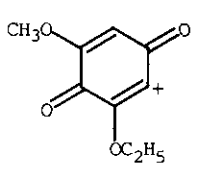
A



B



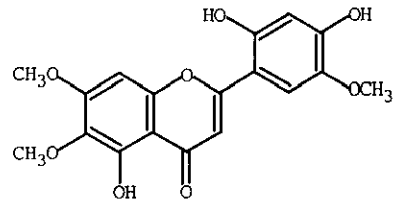
C



D

Accumulated evidence thus established the structure of arcapillin as represented by formula I.

In order to substantiate this conclusion, the ^{13}C NMR spectra of arcapillin and its triacetate (II), prepared by acetylation with acetic anhydride in pyridine, were subjected to examination (Table I). Thus, the observed values in arcapillin were revealed to be in agreement with the calculated values of the signals which were obtained by the use of the two model compounds, 5,2'-dihydroxy-6,7-dimethoxyflavone (IV)[11] for A and C-ring and 5,7,4'-trihydroxy-6,8,3'-trimethoxyflavone (V)[11] for B-ring, coupled with addition of the substitution effects of hydroxyls in a benzene ring.[12] Further, it was found that



I

Table I. Carbon-13 shieldings in arcapillin and its triacetate (δ)

	arcapillin		triacetate	
	obsd.	calcd.	obsd.	calcd.
C-2	163.0 s	161.5	159.0 s	—
C-3	108.3 d	108.6	112.5 d	—
C-4	183.5 s	182.3	176.2 s	—
C-5	153.5 s	151.8	142.1 s	147.9
C-6	132.8 s	132.9	139.9 s	139.1
C-7	159.0 s	158.6	158.0 s	158.1
C-8	91.5 d	91.5	98.2 d	97.1
C-9	155.0 s	151.8	149.5 s	151.3
C-10	106.3 s	105.0	111.4 s	111.2
C-1'	108.6 s	108.8	123.5 s	120.6
C-2'	153.5 s	146.8	141.5 s	142.4
C-3'	105.6 d	103.1	119.0 d	115.5
C-4'	153.5 s	149.6	142.0 s	145.2
C-5'	142.6 s	142.6	154.3 s	154.4
C-6'	112.4 d	111.5	112.3 d	110.5

the observed values determined in the acetate (II) were compatible with the calculated values of the signals obtained on the basis of chemical shifts of the carbons in the same model compounds (IV and V) by considering the substitution effects of hydroxyl and acetoxyl in a benzene ring (Table I). These results confirmed the structure I for arcapillin.[13]

Arcapillin and eupatolitin (0.3-1.0 mg/ml in the culture medium) were shown to exhibit significant protective effects in dose-dependent manners when applied directly to primary cultured liver cells of rat damaged by carbon tetrachloride, the potency being stronger in arcapillin than in eupatolitin.[4]

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NOTES AND REFERENCES

1) Liver-protective drugs. Part 5. This paper also forms Part 42 in the series on the validity of the Oriental medicines.
2) T. Komiya, M. Tsukui and H. Oshio, Yakugaku Zasshi, 1976, **96**, 841.
3) K. Mashimo, K. Shimizu and G. Chihara, Saishin Igaku, 1963, **18**, 1430.
4) Detailed results will be published elsewhere.
5) Unless otherwise specified, UV, IR and ^{1}H NMR spectra were taken in methanol, KBr disks and dimethyl sulfoxide-d_6, respectively. ^{13}C NMR spectra were measured in pyridine-d_5 (arcapillin) and chloroform-d_1 (triacetate).
6) L. Quijano, F. Malanco and T. Rios, Tetrahedron, 1970, **26**, 2851.
7) R. Mues, B. N. Timmermann, N. Ohno and T. J. Mabry, Phytochemistry, 1979, **18**, 1379.
8) T. J. Mabry, K. R. Markham and M. B. Thomas, 'The Systematic Identification of Flavonoids', Springer-Verlag, Heidelberg, 1970, pp. 41-60.
9) J. A. Mears and T. J. Mabry, Phytochemistry, 1972, **11**, 411.
10) D. G. I. Kingston, Tetrahedron, 1971, **27**, 2691.
11) M. Iinuma, S. Matsuura and K. Kusuda, Chem. Pharm. Bull., 1980, **28**, 708.
12) J. B. Stothers, 'Carbon-13 NMR Spectrometry', Academic Press, New York, 1972, p. 197.
13) Isolation of a similar substance was also reported quite recently: T. Namba et al., Abstract of Papers, The 102nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, 1982, p. 587.

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