Chem. Pharm. Bull. 29(6)1777—1779(1981)

Structures of Isoagastachoside and Agastachin, New Glucosylflavones isolated from *Agastache rugosa*

Acacetin, tilianine, and new glucosylflavones, isoagastachoside (2″-O-acetyl tilianine) and agastachin (di-6″-tilianine malonate), were isolated from the aerial part of *Agastache rugosa* (Labiatae). These structures were confirmed by the various spectroscopic evidences.

Keywords——*Agastache rugosa*; Labiatae; flavonoids; acetyl glucosylflavone; malonyl glucosylflavone; isoagastachoside; agastachin; ^{13}C-NMR

Agastache rugosa and *Pogostemon cablin* (Labiatae) are commercially utilized as "KAKKO" (in Japanese) which is one of the crude drugs in Chinese medicine. Since details on the essential oils[1] in these plants have been already reported, studies on their flavonoids have been undertaken and we reported previously on six flavonoids isolated from *P. cablin*.[2] Recently, acacetin (I), tilianine (III), agastachoside (V), and acacetin 7-O-rutinoside (linarin) were isolated from *A. rugosa*.[3] In the course of studies on the quality-evaluation of *A. rugosa*, we isolated two new glucosylflavones, isoagastachoside (II) and agastachin (IV), from this plant at the time of flowering.

The aerial parts of *A. rugosa* (1 kg) were extracted with *n*-hexane and then with methanol. The MeOH extract was dissolved in water, transferred into a separatory funnel, and extracted with ethyl acetate. Chromatographic purification of the AcOEt extract (20 g) furnished compounds I (285 mg), II (210 mg), III (46 mg), and IV (19 mg). Compounds I and III were respectively confirmed as acacetin and tilianine, which were previously isolated from this plant, by their various spectral data.[3,4]

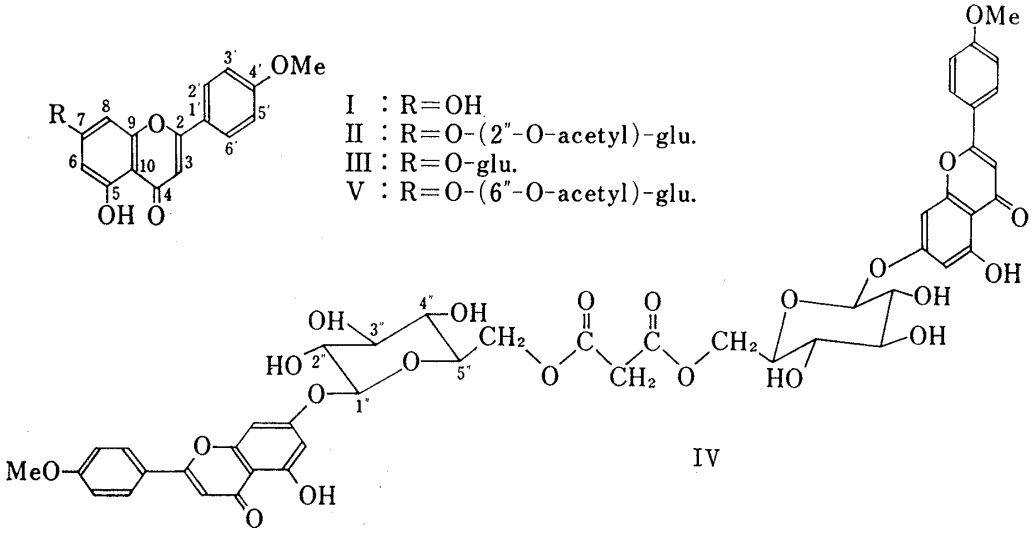

Fig. 1. The Flavonoids isolated from *Agastache rugosa*

Isoagastachoside (II), pale yellowish columns, mp 225—227° (lower layer solvent of $CHCl_3$–MeOH–H_2O (35 : 65 : 40)), *Anal.* Calcd for $C_{24}H_{24}O_{11}\cdot 1/3H_2O$: C, 58.30; H, 5.03. Found: C, 58.34; H, 5.15., UV λ_{max}^{MeOH} nm (ε): 268 (21200), 326 (23800), MS *m/z* (%) : 488 (M^+, 5), 284 (100), 152 (6), 132 (11), gave positive HCl/Mg and HCl/Zn tests. Its fragment pattern was similar to that of acacetin in EI–MS. Upon the alkaline hydrolysis (0.2 N NaOH, stirred for 5 min. at room temperature) of II, III was identified by TLC. The bathochromic shift of UV absorption with $AlCl_3$ suggested the presence of 5-hydroxyl group and the absence of band

TABLE I. ^{13}C Chemical Shifts of Flavonoids isolated from *Agastache rugosa*

Carbon No.	I	II	III	IV	V
2	162.99	162.37	162.83	162.53	162.76
3	103.32	103.67	103.61	103.67	103.78
4	181.51	181.75	181.79	181.16	181.97
5	157.17	156.71	156.77	157.00	156.94
6	98.77	99.29	99.40	99.57, 99.69	99.63
7	164.03	163.68	163.63	163.11	163.75
8	93.87	95.02	94.29	94.56, 94.68	94.79
9	162.07	162.13	162.25	162.13	162.42
10	103.72	105.57	105.22	105.86	105.45
1′	122.70	122.47	122.47	122.52	122.64
2′, 6′	127.94	128.29	128.23	128.17	128.35
3′, 5′	114.28	114.45	114.45	114.45	114.56
4′	161.39	161.09	160.92	161.27	161.15
1″		97.33	99.80	99.80	99.63
2″		73.28	73.00	72.99	73.05
3″		73.69	77.03	75.93	76.28
4″		69.59	69.48	69.83	69.65
5″		77.32	76.28	74.03	73.92
6″		60.62	60.48	63.08	63.48
4′-OMe	55.29	55.47	55.41	55.41	55.58
Acetyl CH_3		20.76			20.64
CO		169.16			169.91
Malonyl CH_2				45.49	
CO				168.65, 169.34	

The measurements were made on a JEOL FX-100 spectrometer in DMSO-d_6 with TMS as an internal reference and are expressed in terms of ppm. The assignments of C-2, 9, and 4′ may be reversed.

II shift with AcONa indicated that the 7-hydroxyl group was substituted. IR ν_{max}^{KBr} cm^{-1}: 1740 and ^1H-NMR (DMSO-d_6) δ: 2.06 (3H, s) of II, suggested the presence of acetyl group. On the basis of the above results, it was assumed that II contained one acetyl group which linked at the glucose moiety of III. Therefore, we attempted to use ^{13}C-NMR method in order to determine the position of acetyl group in the glucose moiety.[5] The ^{13}C-NMR spectral data for acacetin and its derivatives are given in Table I. As can be seen from Table I, it is evident that acetyl group links at the hydroxyl group of C-2″ in the glucose moiety, because C-1″ and C-3″ carbon signals of II are shifted to upfield in comparison with those of III. Consequently, isoagastachoside (II) was established as 2″-O-acetyl tilianine.

Agastachin (IV), a pale yellowish powder, mp 192—195° (lower layer solvent of $CHCl_3$–MeOH–H_2O (35: 65: 40)), UV λ_{max}^{MeOH} nm (ε): 269 (47100), 322 (50400), MS *m/z* (%): 284 (77), 152 (7), 132(14), 44 (100), positive HCl/Mg and HCl/Zn tests, was speculated as a derivative of III on the basis of its various spectral data. When IV was allowed to stand in DMSO solution at room temperature for about one week, it decomposed to III and V, and the latter was established to contain acetyl group linked at the hydroxyl group of C-6″ in the glucose moiety of III by its ^{13}C-NMR spectral elucidation[5] in Table I. When IV in DMSO–H_2O (1: 1) solution was heated for three hours on a water bath, it was convertible into III only. The carbon signals of δ 45.49 (t, CH_2), 168.65 (s, C=O) and 169.34 (s, C=O) except for the signals of glucosylflavone moieties and 1725 cm^{-1} in IR of IV suggested the presence of malonyl diester. This was supported by the fact that IV gave a negative test with the color reagent of bromcresol green in TLC.[6] When the number of each proton in ^1H-NMR spectrum of IV was calculated on the basis of two protons of methylene (DMSO-d_6 δ: 3.35 (s) in ^1H-NMR, and 45.59 (t) in ^{13}C-NMR) of malonic acid moiety, it was confirmed that IV consisted of two molecules of III and one of malonic acid. Furthermore, it was apparent that two carboxyl groups of malonic acid were esterified with the hydroxyl group of C-6″ in each glucose of two tilianines, because

the C-6″ carbon signal of III at δ 60.48 was shifted downfield to δ 63.08 in IV. From the above results, it was indicated the structure of IV to be symmetrical. As can be seen from Table I, the carbon signals of its two glucosylflavone moieties were similar to those in agastachoside (V), and were all equivalent except for the signals of C-6 and C-8. The difference of their chemical shifts is assumed to be due to the nonsymmetrical structure of IV conformationally. Consequently, agastachin (IV) was confirmed as di-6″-tilianine malonate.

On the basis of the fact that malonyl diglucosylflavone, agastachin (IV), was isolated from *A. rugosa*, we speculated that the biosynthetic pathway for compounds II—V would be as follows. The 6″-hydroxyl group of glucosylflavone (III) would be malonylated by malonyltransferase[7] to give malonyl glucosylflavone[8] and then to be transformed into 6″-O-acetyl glucosylflavone (V) by its decarboxylation. The malonyl glucosylflavone would be converted into 6″-malonyl-CoA glucosylflavone by CoA-ligase and furthermore, into malonyl diglucosylflavone (IV) by the condensation with glucosylflavone (III). 2″-Malonyl glucosylflavone which would be biosynthesized by the direct malonylation of III or by the intramolecular condensation with the 2″-hydroxyl group of 6″-malonyl -CoA glucosylflavone in the twist-boat form of its glucose moiety, would furnish 2″-O-acetyl glucosylflavone (II) by the decarboxylation. This assumption is also of interest in connection with biogenetic relationships between 2″- and 6 -O-acetyl glucosylflavonoids, in the form of which O-acetyl glucosylflavonoids were generally isolated from natural sources.[4,9]

Acknowledgement We are grateful to Mr. H. Izumi for the supply of *A. rugosa* and also to Mr. Y. Shida and Mrs. C. Sakuma of the Instrumental Analysis Center of this college for mass and NMR spectral measurements.

References and Notes

1) *P. cablin*; G. Buchi, I.M. Goldman, and D.W. Mayo, *J. Am. Chem. Soc.*, **88**, 3109 (1966); N. Tsubaki, K. Nishimura, and Y. Hirose, *Bull. Chem. Soc. Jpn.*, **40**, 597 (1967); H. Hikino and K. Ito, *Chem. Pharm. Bull.*, **16**, 1608 (1968); S.J. Terhune, J.W. Hogg, and B.M. Laurence, *Tetrahedron Lett.*, **1973**, 4705; E. Trifilie, *Phytochemistry*, **19**, 2467 (1980).
A. rugosa; Y. Fujita and T. Ueda, *Nippon Kagaku Zasshi*, **78**, 1541 (1957); Y. Fujita, T. Ueda, and S. Fujita, *ibid.*, **85**, 892 (1964); Y. Fujita and S. Fujita, *ibid.*, **86**, 635 (1965); S. Fujita and Y. Fujita, *Yakugaku Zasshi*, **90**, 1514 (1970); *idem*, *ibid.*, **92**, 908 (1972).
2) H. Itokawa, K. Suto, and K. Takeya, *Chem. Pharm. Bull.*, **29**, 254 (1981).
3) O.I. Zakharova, A.M. Zakharova, and V.I. Glyzin, *Khim. Prir. Soedin*, 642 (1979).
4) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer, New York, 1970; J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids," Chapman and Hall, London, 1975.
5) K. Yoshimoto, Y. Itatani, and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980).
6) D. Braun and H. Geenen, *J. Chromatogr.*, **7**, 56 (1962).
7) F. Kreuzaler and K. Hahlbrock, *Phytochemistry*, **12**, 1149 (1973).
8) A.B. Beck and J.R. Knox, *Aust. J. Chem.*, **24**, 1509 (1971).
9) C. Redaelli, L. Formentini, and E. Santaniello, *Phytochemistry*, **19**, 958 (1980).

Department of Pharmacognosy,
Tokyo College of Pharmacy,
Horinouchi 1432-1, Hachioji,
Tokyo 192-03, Japan

Hideji Itokawa*
Keiichi Suto
Koichi Takeya

Received March 18, 1981