Phytochemistry, Vol. 21, No. 6, pp. 1466–1468, 1982.
Printed in Great Britain.

0031-9422/82/061466-03$03.00/0

RHAMNUSTRIOSIDE, A FLAVONOL TRIGLYCOSIDE FROM *RHAMNUS NAKAHARAI**

CHUN-NAN LIN, MUNEHISA ARISAWA,† MINEO SHIMIZU† and NAOKATA MORITA†

School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China; †Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Shugitani 2630, Toyama, Japan

(*Revised received* 20 *August* 1981)

Key Word Index—*Rhamnus nakaharai*; Rhamnaceae; flavonol triglycoside; rhamnustrioside; kaempferol 4′-*O*-rhamnosyl-(1→2)-*O*-[rhamnosyl-(1→6)]-galactoside; branched trisaccharide.

Abstract—A new flavonol triglycoside, rhamnustrioside, has been isolated from the fresh fruits of *Rhamnus nakaharai* together with kaempferol. The structure of rhamnustrioside has been determined as kaempferol 4′-*O*-rhamnosyl-(1→2)-*O*-[rhamnosyl-(1→6)]-galactoside, by chemical degradation and spectral means.

INTRODUCTION

Rhamnus nakaharai Hayata is endemic to southern and northern parts of Taiwan, in forests at about 1300 m [1]. The fresh fruit on extraction yielded kaempferol and a new flavonol triglycoside, rhamnustrioside, the structural determination of which is described in this paper.

RESULTS AND DISCUSSION

Rhamnustrioside, $C_{33}H_{40}O_{19}$, was recognized as a flavonol glycoside from colour reactions and its spectral properties; on acid hydrolysis it afforded kaempferol, galactose and rhamnose. The ^{1}H NMR spectrum (CCl_4) of the trimethylsilyl (TMS) ether of rhamnustrioside showed a pair of 2H doublets (J = 9 Hz), centered at δ 8.08 and 7.08, assignable to the proton located at the 2′,6′ and 3′,5′-positions and a pair of 1H doublets (J = 2 Hz), centered at δ 6.45 and 6.15, assignable to the proton located at the 8- and 6-positions, respectively. The 1H doublets (J = 7 Hz), centered at δ 4.88, was attributed to the galactosyl anomeric proton and a pair of 1H doublets (J = 2 Hz), centered at δ 4.70 and 4.50, to the two rhamnosyl anomeric protons respectively [2]. Signals of 14H at δ 3.42–4.07 were attributed to the aliphatic protons. The 6H broad singlet at δ 1.17 indicated two rhamnosyl methyl groups [2] and these data clearly show that rhamnustrioside has 2 mol of rhamnose. Hydrolysis of rhamnustrioside permethylate with hydrochloric acid afforded kaempferol 3,5,7-trimethyl ether, confirming rhamnustrioside as a kaempferol 4′-triglycoside.

Acetylation of rhamnustrioside afforded a colourless acetate $C_{55}H_{62}O_{30}$. The ^{1}H NMR spectrum ($CDCl_3$) of the acetate (see Experimental) also confirmed the structural assignment of rhamnustrioside as above. In the mass spectrum of the acetate, an intense peak of m/z 273 indicated the sugar sequence: aglycone–galactose–rhamnose [3].

In the ^{13}C NMR spectrum of kaempferol-3-galactoside [4], signals at 101.9, 71.3, 68.0, 75.7, and 60.3 ppm belong to galactosyl C_1, C_2, C_3, C_4, C_5, and C_6 respectively. In that of kaempferol-3-rhamnoside [5], the signal at 101.5 ppm is attributed to rhamnosyl C_1. Signals at 71.1 and 70.3 ppm are attributed to rhamnosyl C_2, C_3, C_4, and C_5 and at 17.8 ppm to rhamnosyl C_6. Kaempferol 3-robinoside-7-rhamnoside [6] has signals at 102.0, 71.3, 73.1, 68.4, 73.8, and 65.6 ppm attributed to galactosyl C_1, C_2, C_3, C_4, C_5, and C_6 respectively. In the ^{13}C NMR spectrum of rhamnustrioside, signals at 102.2, 77.9, 72.9, 68.3, 73.6, and 66.0 ppm were attributed to galactosyl C_1, C_2, C_3, C_4, C_5, and C_6 [4, 6]. The chemical shift of rhamnustrioside on galactosyl C_2 with 6.6 ppm shifted to low field when compared with that of kaempferol-3-galactoside and kaempferol-3-robinoside-7-rhamnoside and on galactosyl C_6 with 5.7 ppm shifted to low field when compared with that of kaempferol-3-galactoside. The signal at 100.2 ppm was attributed to rhamnosyl C_1, signals at 70.3 and 72.1 ppm to rhamnosyl C_2, C_3, C_4, and C_5 and the signal at 17.8 ppm to rhamnosyl C_6. The chemical shift to the rhamnosyl carbon of rhamnustrioside was identical with that of kaempferol-3-rhamnoside. The chemical shifts of the aglycone of rhamnustrioside (Table 1) were identical with those of kaempferol. Thus rhamnustrioside was identified as kaempferol 4′-*O*-rhamnosyl-(1 → 2)-*O*-[rhamnosyl-(1 → 6)]galactoside.

EXPERIMENTAL

All mps were uncorr. UV spectra were determined in MeOH and IR spectra recorded in KBr. NMR spectra were measured at 60 MHz with TMS as int. standard. ^{13}C NMR spectra were obtained on a JEOL FX-60 spectrometer recorded in combination with a JEC-6 Spectrum computer.

*Part 1 in the series "The Constituents of Formosan *Rhamnus* Species".

Table 1. ^{13}C chemical shift values and assignment*

Carbon number	1	2	Flavonols 4	5	6
Galactosyl					
1	—	102.2	101.9	—	102.0
2	—	77.9	71.3	—	71.3
3	—	72.9	73.1	—	73.2
4	—	68.3	68.0	—	68.4
5	—	73.6	75.7	—	73.8
6	—	66.0	60.3	—	65.6
Rhamnosyl					
1	—	100.2	—	101.5	100.1
2	—		—		70.5
3	—	[70.3	—	[70.3	70.5
4	—	72.1]	—	71.1]	72.1
5	—		—		68.4
6	—	17.8	—	17.8	18.1
Flavonol skeleton					
2	146.7	156.0	156.4	157.2	—
3	135.7	136.0	133.4	134.2	—
4	175.8	175.8	177.5	177.7	—
5	160.7	160.5	161.1	161.3	—
6	98.2	98.2	98.8	98.9	—
7	163.8	163.8	164.2	164.2	—
8	93.4	93.6	93.8	93.7	—
9	156.2	156.0	156.4	156.4	—
10	103.0	103.0	104.0	104.1	—
1′	121.7	124.3	120.9	120.5	—
2′	129.5	129.1	131.0	130.6	—
3′	115.4	116.0	115.1	115.3	—
4′	159.2	158.3	159.9	160.0	—
5′	115.4	116.0	115.1	115.3	—
6′	129.5	129.1	131.0	130.6	—

In DMSO-d_6, δ: ppm, TMSO.
*See refs. [7, 8].

Chemical shifts were recorded as δ values (ppm) with TMS as int. standard.

Extraction and separation. The fresh fruits of *Rhamnus nakaharai* (0.85 kg), were collected at Ali, Wu-Tai Shian, Ping-Tung Hsien, Taiwan, during July 1977, chipped and extracted with hot MeOH. The MeOH extract was divided into hot H_2O soluble and insoluble fractions. Kaempferol and rhamnustrioside were obtained from the Et_2O extract of the former fraction and the Me_2CO insoluble portion of the latter fraction, respectively.

Kaempferol. Recrystallization from MeOH gave yellow needles, mp 276–278°. It was proved to be kaempferol by mmp and comparison of IR spectra with an authentic sample.

Rhamnustrioside. Recrystallization from MeOH + Me_2CO gave yellow needles, mp 206–207°, yellow in UV light. PC R_f 0.45 (15% HOAc), 0.56 (30% HOAc). Found: C, 53.71; H, 5.54. $C_{33}H_{40}O_{19}$ requires: C, 53.49; H, 5.45. UV λ_{max} nm (log ϵ): 267 (4.16), 316 (3.95), 363 (4.18); +$AlCl_3$: 269, 305 (sh), 343, 419; +NaOAc: 275, 310 (sh), 379. IR ν_{max} cm^{-1}: 1660 (>CO). ^1H NMR: (TMS ether of rhamnustrioside, CCl_4, 60 MHz): δ 1.17 (6H, *br s*, rhamnosyl 2-Me), 3.42–4.07 (14H, *br* aliphatic H), 4.50 (1H, *d*, $J = 2$ Hz, rhamnosyl anomeric H), 4.70 (1H, *d*, $J = 2$ Hz, rhamnosyl anomeric H), 4.88 (1H, *d*, $J = 7$ Hz, galactosyl anomeric H), 6.15 (1H, *d*, $J = 2$ Hz, H-6), 6.45 (1H, *d*, $J = 2$ Hz, H-8) 7.08 (2H, *d*, $J = 9$ Hz, H-3′, H-5′), 8.08 (2H, *d*, $J = 9$ Hz, H-2′, H-6′). ^{13}C NMR: see Table 1.

Rhamnustrioside acetate. This was a colourless powder. Found: C, 54.82; H, 5.27. $C_{55}H_{62}O_{30}$ requires: C, 54.89; H, 5.20. ^1H NMR: ($CDCl_3$, 60 MHz): δ 1.17 (6H, *d*, $J = 6$ Hz, rhamnosyl 2 Me), 1.99, 2.03, 2.09, 2.13, and 2.20 (total 24H, aliphatic 8 OAc), 2.33 (6H, *s*, aromatic 20Ac), 2.46 (3H, *s*, aromatic OAc), 3.47–4.07 (7H, *br*, aliphatic H), 4.73–5.50 (10H, *br*, aliphatic H), 6.87 (1H, *d*, $J = 2$ Hz, H-6), 7.09 (2H, *d*, $J = 9$ Hz, H-3′, H-5′), 7.37 (1H, *d*, $J = 2$ Hz, H-8), 7.83 (2H, *d*, $J = 9$ Hz, H-2′, H-6′). MS (*m/*): 503, 328, 287, 286, 273, 213, 171, 153, 111.

4′-Hydroxy-3,5,7-trimethoxyflavone (*kaempferol-3,5,7-trimethyl ether*). Rhamnustrioside (100 mg) in dry Me_2CO (100 ml) was refluxed over dry K_2CO_3 (5 g) with dry Me_2SO_4 (1 ml) for 12 hr. The product was heated with 1 N HCl and yielded colourless needles, mp 282–283°, ex. MeOH. UV λ_{max} nm: 256 (*s*), 262, 336; +$AlCl_3$: unchanged; +NaOAc: unchanged; +NaOMe: 264, 259 (*s*), 378. ^1H NMR: (DMSO-d_6, 60 MHz): δ 3.23 (1H, *s*, phenolic OH), 3.70 (3H, *s*, OMe),

3.80 (3H, *s*, OMe), 3.85 (3H, *s*, OMe) 6.42 (1H, *d*, $J = 2$ Hz, H-6), 6.71 (1H, *d*, $J = 2$ Hz, H-8), 6.89 (2H, *d*, $J = 9$ Hz, H-3', H-5'), 7.90 (2H, *d*, $J = 9$ Hz, H-2', H-6').

Acknowledgements—We are indebted to Dr. H. C. Hsieh, President of Kaohsiung Medical College and Professor S. T. Lu, Kaohsiung Medical College, for their encouragement; Associate Professor T. Okuyama, Meiji College of Pharmacy, for ^{13}C NMR measurement, and Messrs M. Morikoshi and H. Hori, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, for mass spectral measurement and elemental analysis, respectively.

REFERENCES

1. Tang-Shui Liu. (1962) *Illustrations of Native and Introduced Ligneous Plants of Taiwan*, p. 832. National Taiwan University, Taipei.
2. Mabry, T. J., Markham, K. R. and Thomas M. B. (1970) *The Systematic Identification of Flavonoids*, p. 269. Springer, New York.
3. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 209. Holden-Day, San Francisco.
4. Matsuura, S. and Iinuma, M. (1978) *Chem. Pharm. Bull.* **26**, 1936.
5. Morita, N. Arisawa, M., Nagase, M., Hsu, H.-Y. and Chen, Y.-P. (1977) *Yakugaku Zasshi* **97**, 649.
6. Wenkert, E. and Gottlieb, H. E. (1977) *Phytochemistry* **16**, 1813.
7. Ternai, B. and Markham, K. R. (1976) *Tetrahedron* **32**, 545.
8. Markham, K. R., Ternai, B., Stanley, R., Geeger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.

Phytochemistry, Vol. 21, No. 6, pp. 1468–1470, 1982.
Printed in Great Britain.

0031-9422/82/061468-03$03.00/0

PRENYLATED FLAVONOIDS FROM *TEPHROSIA PURPUREA* SEEDS*

BHARATI SINHA, A. A. NATU and D. D. NANAVATI

National Chemical Laboratory, Pune 411 008, India

(*Revised received* 13 *October* 1981)

Key Word Index—*Tephrosia purpurea*; Leguminosae; purpuritenin; purpureamethide; pongamol; karanjin; lanceolatin B; prenylated flavonoids; methylated chalcone.

Abstract—Two new prenylated flavonoids, purpuritenin and purpureamethide, have been characterized from the seeds of *Tephrosia purpurea* together with the known compounds pongamol, karanjin and lanceolatin B.

Tephrosia Pers. (Galegeae, Lotoideae, Leguminosae) is a large tropical and sub-tropical genus of some 300 species[1]. Earlier phytochemical screening [2] of a number of species have revealed the presence of rotenoids, isoflavones, flavanones, chalcones, flavonols and flavones. Within the group of flavones, 5, 7-oxygenated and 7-oxygenated compounds which are characterized by the presence of a C-8 prenyl unit are well known. In many cases, these prenylated flavones have undergone further substitution and cyclization leading to complex molecules. *T. purpurea* Pers. occurs throughout the Indian subcontinent. This species has been reported to contain a number of rotenoids [3] besides pongamol [4], isolonchocarpin[5], karanjin, lanceolatin B, kanjone and sitosterol[6]. Recent reports[7] indicating insecticidal and repellent properties of the seed extract of this plant prompted us to undertake a study of the active principle from this species. We now report the occurrence of five flavonoids; pongamol (**1**), karanjin (**2**), lanceolatin B (**3**) and two new compounds purpuritenin (**4**) and purpureamethide (**5**) (Fig. 1) from the seeds of *T. purpurea*.

Pongamol (**1**) was identified by complete spectral analysis (UV, IR, ^1H NMR, MS) and comparison with an authentic sample. Karanjin (**2**) and lanceolatin B (**3**) were also characterized by spectral data and comparison with authentic samples.

Purpuritenin (**4**) was analysed for $C_{19}H_{16}O_3$ ([M]$^+$ 292), $[\alpha]_D^{CHCl_3} \pm 0°$. The structure was assigned on the basis of spectral data. ^1H NMR of **2** was very similar to ovalitenin A isolated from *Milletia ovalifolia*[8] except for an additional aromatic methyl group in the former compound. Alkaline hydrolysis of **4** gave *p*-toluidic acid, mp 182–183°, thus confirming *para*-substitution in ring A with a methyl at C-4 and the structure of purpuritenin as 4-methyl-2'-methoxy-(3',4',2'',3'')furano-chalcone. Prenylated flavonoids [2] are very well known in the literature but to our knowledge, this is a rare example of a methylated

*NCL Communication No. 2823.