TRADITIONAL MEDICINAL PLANTS OF THAILAND. I. ISOLATION AND STRUCTURE ELUCIDATION OF TWO NEW FLAVONOIDS, (2*R*,3*R*)-DIHYDROQUERCETIN-4'-METHYL ETHER AND (2*R*,3*R*)-DIHYDROQUERCETIN-4',7-DIMETHYL ETHER FROM *BLUMEA BALSAMIFERA*

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ABSTRACT.—The leaves of *Blumea balsamifera* (Compositae) have yielded two new flavonoids, the 4'-methyl ether (**1a**) and the 4',7-dimethyl ether (**2a**) of 2*R*, 3*R*-dihydroquercetin. The structures were deduced by spectral interpretation and chemical correlation.

Blumea balsamifera DC is a member of the tribe Inuleae (Compositae) which has been used in the traditional medicine of some Oriental cultures. The Chinese have used preparations of this plant as a carminative, a mild stimulant, a vermifuge, as a topical application for septic ulcers, and as a preventive medicament in times of epidemics. The ancient Chinese medical literature has recorded its use as an abortifacient (1).

Preparations of *Blumea* are presently finding use in the traditional medicine of Thailand, where they are available at local herbal drug shops. Cigarettes are prepared from the chopped, dried leaves of *B. balsamifera* and smoked to relieve the pain of sinusitis. An infusion prepared from leaf material is used as a stomachic, carminative, diaphoretic, expectorant and emmenagogue. A decoction of fresh leaves is used, alone or in combination with other plant preparations, as a bath for women following childbirth.

The chemistry of *Blumea* constituents has been of some interest for at least 65 years. The essential oil was the first product to be studied (2). In this initial study, *d*-carvotanacetone, *l*-tetrahydrocarvone, a mixture of butyric, isobutyric and *n*-octanoic acids, and an unidentified phenol were isolated. Subsequently, *l*-borneol (1), fenchone (3), 1,8-cineol (3), two carvotanacetone derivatives (4), a diester of coniferyl alcohol (5), some polyacetylenes and thiophene derivatives (5, 6), campesterol (7), stigmasterol (8), sitosterol (9), xanthoxylin (1), erianthin and 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (3, 10–12), other unidentified flavonoids (1, 10), coumarins and triterpenes (1), and myristic acid (3) have been isolated from *Blumea* species.

The present study was undertaken in order to expand our knowledge of the constituents of this medicinally interesting genus, and describes the isolation of two flavonoid derivatives, (2*R*,3*R*)-dihydroquercetin-4'-methyl ether (**1a**) and (2*R*,3*R*)-dihydroquercetin-4',7-dimethyl ether (**2a**), both of which are new natural products.

EXPERIMENTAL[1]

PLANT MATERIAL.—Leaf material of *Blumea balsamifera* DC (Compositae) was purchased from a local herbal drug shop in Bangkok and authenticated by comparison with herbarium specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. This material was then air dried and coarsely powdered.

EXTRACTION AND INITIAL FRACTIONATION.—The air dried and powdered plant material (4.5 kg) was macerated twice for 5 day periods with 95% ethanol (20 and 15 liters). The ethanol extracts were pooled, the alcohol removed *in vacuo*, and the residue suspended in 3 liters of 10% ethanol. After filtration, the filtrate was treated with a 10% aqueous lead acetate solution until no further precipitation occurred. Further filtration then afforded a clear solution which was extracted with chloroform (3 x 7 liters). The combined chloroform extract was dried (Na_2SO_4) and then evaporated *in vacuo* to yield 13.5 g of a brown syrupy mass which, when stirred with chloroform (90 ml), produced a pale yellow precipitate. Recovery by filtration and drying yielded a solid (Fraction A, 977 mg) and a filtrate which was evaporated *in vacuo* to a yellow syrup (Fraction B, 12 g).

SEPARATION OF FRACTION A.—Thin layer chromatography (tlc) of Fraction A (silica gel G[2], chloroform: acetone (9:1)) indicated the presence of only two components (Rf=0.17 and 0.35). The total fraction was divided into four portions. Each portion was dissolved in chloroform (2 ml), adsorbed onto silica gel (5 g), dried, then placed on top of a dry silica gel 60[2] column (2.5 x 40 cm), and eluted with chloroform-acetone (9:1). Fractions of 20 ml each were collected and compared by tlc. Those fractions of similar composition were combined. This procedure produced 3 major fractions which were evaporated *in vacuo*. Fractions 1–5 yielded no residue on evaporation; fractions 6–10 were homogeneous by tlc and were designated PS-2; fractions 11–19 were also homogeneous by tlc and were designated PS-1.

(2*R*,3*R*)-DIHYDROQUERCETIN-4'-METHYL ETHER (**1a**).—Fraction PS-1, when evaporated to dryness *in vacuo*, yielded 766 mg (1.7 x 10^{-4}%) of a light yellow, amorphous powder, mp 173–4°; $[\alpha]^{24}D$ +14.9°; ir, νmax (KBr), 3400, 1645, 1600, 1280, and 1160 cm^{-1}: uv, data summarized in table 1; cd (MeOH), $[\theta]_{328}$ = +3.35 x 10^5, $[\theta]_{293.5}$ = −1.31 x 10^5, $[\theta]_{253.5}$ = +1.95 x 10^5, $[\theta]_{220}$ = +1.44 x 10^6 deg·cm^2/dmole; ^{1}H-nmr (DMSO-d_6) δ, 3.78 (3H, s, $ArOCH_3$), 4.48 (1H, bd, *J*=11 Hz, 3-H), 3.81 (3H, m, 3 x OH), 5.03 (1H, d, *J*=11 Hz, 2-H), 5.87 (2H, AB system, 6-H and 8-H), 6.91 (3H, bm, 2'-H, 5'-H and 6'-H), and 11.88 (1H, bs, OH); ^{1}H-nmr (acetone-d_6) δ, 3.88 (3H, s, $ArOCH_3$), 4.61 (1H, d, *J*=11.5 Hz, 3-H), 5.08 (1H, d, *J*=11.5 Hz, 2-H), 5.98 (2H, AB system, 6-H and 8-H), 7.00–7.10 (3H, m, 2'-H, 5'-H and 6'-H), and 11.68 (1H, bs, OH); ms, *m/z* 318 (M^-, 37%, $C_{16}H_{14}O_7$), 289 (44), 166 (50), 165 (26), 164 (36), 153 (100), and 137 (39).

(2*R*,3*R*)-DIHYDROQUERCETIN-4',7-DIMETHYL ETHER (**2a**).—Fraction PS-2, when evaporated to dryness *in vacuo*, yielded 94 mg (2.1 x 10^{-5}%) of a light yellow, amorphous powder, mp 164–7°; $[\alpha]^{24}D$ +14.8°; ir, νmax (KBr), 3480, 1630, 1510, 1273, 1150, and 1130 cm^{-1}; uv, data summarized in table 1; cd (MeOH), $[\theta]_{331.5}$ = +3.59 x 10^5, $[\theta]_{294}$ = −1.26 x 10^6, $[\theta]_{253.5}$ = +1.71 x 10^5, $[\theta]_{221.5}$ = +1.28 x 10^6 deg·cm^2/dmole; ^{1}H-nmr (DMSO-d_6) δ, 3.79 (6H, bs, 2 x $ArOCH_3$), 4.55 (1H, m, 3-H), 5.09 (1H, d, *J*=11 Hz, 2-H), 6.10 (2H, AB system, *J*=2 Hz, 6-H and 8-H), and 6.95 (3H, m, 2'-H, 5'-H and 6'-H): ^{1}H-nmr (acetone-d_6) δ, 2.78 (3H, bm, 3 x OH), 3.86 (3H, s, $ArOCH_3$), 3.87 (3H, s, $ArOCH_3$), 4.62 (1H, d, *J*=11.6 Hz, 3-H), 5.11 (1H, d, *J*=11.6 Hz, 2-H), 6.07 (2H, AB system, 6-H and 8-H), and 7.00–7.10 (3H, m, 2'-H, 5'-H and 6'-H); ms, *m/z* 332 (M^+, 25%, $C_{17}H_{16}O_7$), 303 (36), 179 (16), 167 (100), 166 (29), 164 (35), 151 (20), and 137 (23).

OXIDATION OF **1a**.—A sample (15 mg) of **1a** was suspended in 2N H_2SO_4 (5 ml) and heated on a steam bath under a gentle stream of air for 24 hours. After the sample was cooled to room temperature and extracted with ethyl acetate (4 x 5 ml), partition was effected against a saturated aqueous solution of sodium bicarbonate to remove residual acid. The organic phase, when dried (Na_2SO_4) and evaporated *in vacuo*, yielded 14.2 mg (95%) of a dark yellow, waxy solid, **3a**; uv, λmax (MeOH), 369, 292, 270 sh, 255 and 205 nm; λmax (MeOH+$NaOCH_3$), 388, 326, 277 and 205 nm, with no change for at least 24 hours.

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[1]Melting points were determined on a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G spectrophotometer. The ir spectra were determined with a Perkin Elmer model 283 spectrophotometer; adsorption bands are reported in wave numbers (cm^{-1}). ^{1}H-nmr spectra were recorded with a Varian T-60A instrument operating at 60 MHz, with a Nicolet Model TT-7 Fourier Transform attachment. Tetramethylsilane was used as an internal standard, and chemical shifts are reported in δ (PPM). Mass spectra were obtained with a Varian MAT-112S double-focusing spectrometer operating at 80 eV and 220°. Optical rotations were obtained in methanol with a Perkin Elmer model 241 polarimeter. CD spectra were obtained in methanol with a JASCO model J-40A automatic recording spectropolarimeter.

[2]E. Merck, Darmstadt, W. Germany.

TABLE 1. Ultraviolet spectra data of PS-1 and PS-2[a]

	PS-1 (**1a**)		PS-2 (**2a**)	
MeOH	327 sh	(log ϵ=3.89)	327 sh	(3.76)
	290	(4.48)	287	(4.49)
	235sh	(4.54)	230sh	(4.57)
	206	(4.82)	216sh	(4.68)
			205	(4.81)
$NaOCH_3$	327	(4.62)	327sh	(3.76)
	290sh	(4.03)	290	(4.47)
	250sh	(4.09)	230sh	(4.57)
	206	(4.82)	217sh	(4.70)
			207	(4.79)
NaOAc	327	(4.58)	327sh	(3.76)
	294sh	(4.19)	287	(4.49)
$NaOAc+H_3BO_3$	327sh	(3.89)	327sh	(3.76)
	290	(4.48)	287	(4.49)
$AlCl_3$	381	(3.92)	384	(3.92)
	315	(4.55)	315	(4.57)
	282	(4.11)	287sh	(4.11)
	223	(4.64)	225	(4.70)
	206	(4.76)	206	(4.92)
$AlCl_3+HCl$	377	(4.01)	384	(3.89)
	315	(4.55)	312	(4.50)
	282	(4.25)	287sh	(4.22)
	223	(4.64)	225	(4.68)
	206	(4.77)	206	(4.91)

[a]All UV spectra were recorded using the standard procedures given in reference 13.

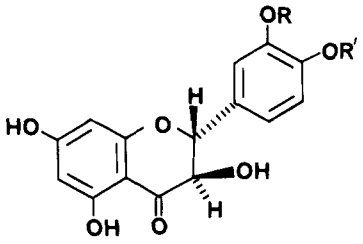
TRIMETHYLSILYLATION OF **3a**.—A sample (14 mg) of **3a** was reacted with TRI-SIL[3] (3 ml) for 15 minutes. The solvent and excess reagent, when removed under vacuum (oil pump) at room temperature, yielded 22 mg (82%) of a crude product which displayed ^{1}H-nmr (CCl_4) δ, 3.87 (3H, s, $ArOCH_3$), 6.13 (1H, d, J=2 Hz, 6-H), 6.29 (1H, d, J=2 Hz, 8-H), 6.84 (1H, d, J=8.5 Hz, 5'-H), and 7.57-7.75 (2H, m, 2'-H and 6'-H).

OXIDATION OF **2a**.—A sample (15 mg) of **2a** was suspended in 2N H_2SO_4 (5 ml) and heated on a steam bath under a gentle stream of air for 48 hours. After the sample was cooled to room temperature and extracted with ethyl acetate (4 x 5 ml), partition was effected against a saturated aqueous solution of sodium bicarbonate to remove residual acid. The organic phase, when dried (Na_2SO_4) and evaporated *in vacuo*, yielded 12.4 mg (83%) of a yellow waxy solid, **4a**; uv, λmax (MeOH), 364, 288, 260, 213 and 208 nm; λmax (MeOH+$NaOCH_3$), 413, 333, 290, 230 and 211 nm, with no change for at least 24 hours.

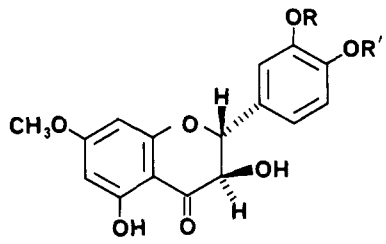
STRUCTURE ELUCIDATION OF **1a** AND **2a**.—The proton nmr data of isolate PS-1 strongly suggested that it was a dihydroflavonol derivative, and the characteristic 11 Hz doublets at 4.48 and 5.03 ppm indicated a *trans*-diaxial arrangement of 2-H and 3-H in such a system. That the signal for 3-H was further split and, on addition of D_2O, sharpened to a doublet indicated that the hydroxyl at C-3 was not methylated (13a). The AB system centered at 5.87 ppm, showing *meta* coupling of approximately 1 Hz, suggested that they were protons at C-6 and C-8 in a dihydroflavonol oxygenated at C-5 and C-7 (13b).

The pronounced bathochromic shift of the band II absorption in the uv spectrum of PS-1 on addition of sodium acetate indicated the presence of a free hydroxyl at C-7 (13c). Addition of aluminum chloride reagent produced bathochromic shifts of band I (54 nm) and band II (25 nm) characteristic of a second free hydroxyl at C-5 (13d). Mass spectral peaks at m/z 166 and 137 substantiated that the remaining hydroxyl and the methoxyl group were indeed on the B-ring (14, 15).

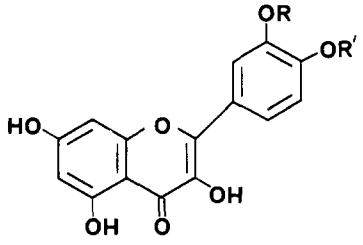
[3]Pierce Chemicals, Rockford, Illinois, USA.



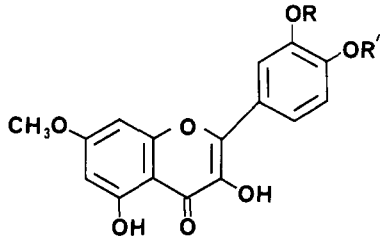
1a R = H, R′ = CH_3
1b R = CH_3, R′ = H



2a R = H, R′ = CH_3
2b R = CH_3, R′ = H



3a R = H, R′ = CH_3
3b R = CH_3, R′ = H



4a R = H, R′ = CH_3
4b R = CH_3, R′ = H

The multiplet at 6.91 (DMSO) or 7.00–7.10 (acetone) in the nmr spectrum of PS-1 are suggestive of oxygenation of C-3′ and C-4′ (13e). However, definitive assignment of the substitution pattern could not be made on the basis of these spectra. A portion of the isolate was oxidized (2N H_2SO_4/air) (16) to the corresponding flavonol, which should be either tamarixetin (**3a**) or isorhamnetin (**3b**). Comparison of the nmr spectrum of the TMS ether of this unknown flavonol with published spectra of the two reference compounds (13f) showed that the unknown flavonol corresponded to tamarixetin (**3a**). This was further substantiated by the stability of the uv spectrum of the oxidation product in the presence of sodium methoxide. Flavonols having a free hydroxyl at C-4′ are unstable in sodium methoxide solution, and their uv spectra degenerate in a few minutes (13g). The flavonol produced by the oxidation of PS-1 was seen to be stable in the presence of sodium methoxide for at least 24 hours. Also, tlc comparison of the oxidation product with isorhamnetin (**3b**) showed them to be different in several systems. A sample of tamarixetin was, unfortunately, not available.

The absolute stereochemistry at C-2 and C-3 was determined by means of circular dichroism. Comparison of the cd curves of PS-1 with those of dihydroflavonols of known configuration (17) established the absolute configuration as 2*R*,3*R*, the signs of the Cotton effects from 400 to 200 nm being respectively +, −, +, +. Thus PS-1 (**1a**) is assigned the structure (2*R*,3*R*,)-dihydroquercetin-4′-methyl ether (or (2*R*,3*R*)-dihydrotamarixetin).

The nmr spectrum of the isolate PS-2 was identical to that of PS-1 except that it showed the loss of one exchangeable proton and the addition of a second aromatic methoxyl group. Since the uv spectrum was unchanged on addition of sodium acetate, this methoxyl group is located at C-7 (13c). Mass spectral evidence confirmed that the additional 14 mass units were indeed in the A-ring. Sulfuric acid oxidation of PS-2 was used to establish the B-ring substitution pattern as in **2a** rather than **2b**. Thus, the uv spectrum of the oxidation product **4a** was found to be quite stable in sodium methoxide solution, and tlc comparison with a reference sample of rhamnazin (**4b**) showed **4a** to be different in several systems. Circular dichroism again established the absolute configuration to be 2*R*,3*R*. Thus, PS-2 (**2a**) is assigned the structure (2*R*,3*R*)-dihydroquercetin-4′,7-dimethyl ether.

In 1972, Herz *et al.* (18) reported the isolation of a dihydroflavonol from a *Eupatorium* hybrid (Compositae) having either **2a** or **2b** as its structure. They were unable to completely define their isolate, having lost the remaining sample in an unsuccessful oxidation reaction.

Comparison of the aromatic region of the nmr spectrum (in DMSO) of their isolate with that of PS-2 showed them clearly to be different. Therefore, from the data presented in ref. 18 and comparison with our isolate, PS-2, we can now propose the structure of the *Eupatorium* isolate as (2*R*,3*R*)-dihydroquercetin-3^1,7-dimethyl ether (**2b**), which is also a previously unreported natural product.

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