Phytochemistry, Vol. 21, No. 3, pp. 805–806, 1982.
Printed in Great Britain.

0031-9422/82/030805-02$03.00/0

FLAVONOIDS OF *GARDENIA CRAMERII* AND *G. FOSBERGII* BUD EXUDATES

A. A. LESLIE GUNATILAKA,* SARATH R. SIRIMANNE, SUBRAMANIAM SOTHEESWARAN* and H. T. BADRA SRIYANI

Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

(*Received* 13 *April* 1981)

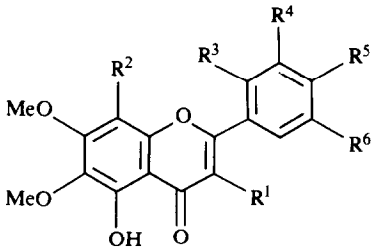
Key Word Index—*Gardenia cramerii*; *G. fosbergii*; Rubiaceae; bud exudates; 5,5'-dihydroxy-6,7,2',3'-tetramethoxyflavone; 5,3',5'-trihydroxy-3,6,7,4'-tetramethoxyflavone; 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone.

Abstract—From the bud exudates of *Gardenia cramerii* and *G. fosbergii*, two species endemic to Sri Lanka, a new flavonoid with an unusual B-ring oxidation pattern, 5,5'-dihydroxy-6,7,2',3'-tetramethoxyflavone, was characterized. Two other rare flavonoids, 5,3',5'-trihydroxy-3,6,7,4'-tetramethoxyflavone and 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone were also isolated from both *Gardenia* species.

In continuation of our studies [1] on the flavonoids of *Gardenia* bud exudates, we now report the isolation and characterization of several polyoxygenated flavonoids from two *Gardenia* species endemic to Sri Lanka namely, *G. cramerii* and *G. fosbergii*.

The hot chloroform extract of the bud exudate of *G. cramerii* was separated into Na_2CO_3 soluble and insoluble fractions. The former contained terpene acids and flavonoids. Terpene acids were removed by washing with 10% borax solution. The borax-insoluble and Na_2CO_3-insoluble fractions on repeated preparative TLC gave three pigments. The least polar pigment was identified as 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone (**1**). The most polar pigment was identical with 5,3',5'-trihydroxy-3,6,7,4'-tetramethoxyflavone (**2**). Both these flavonoids are rare and have been reported previously by us from *G. fosbergii* [1]. The next polar pigment, mp 218° was found to be a new flavonoid, **3**, which had the molecular formula, $C_{19}H_{18}O_8$ (high-resolution mass spectrometry). The UV and IR data were characteristic of a flavone. The ^1H NMR data indicated it to be a tetramethoxy compound with a chelated hydroxyl group. The aromatic region in the NMR spectrum of **3** in acetone-d_6 had singlets at δ 7.2(2H), 6.85(1H) and 6.75(1H) whereas in $CDCl_3$ it had two singlets at δ 6.6(1H), 6.53(1H) and doublets at δ 6.9(1H), 7.2(1H). The doublets were found to be due to a *meta*-coupled aromatic system ($J = 2$ Hz). Comparison of these data with the NMR data in $CDCl_3$ of other flavonoids isolated by us earlier [1] showed that the singlets at δ 6.6(1H) and at δ 6.53(1H) were due to the protons at C-8 and C-3, respectively, of the flavonoid moiety. These data indicate that the A-ring of the flavonoid is oxygenated at C-5, C-6 and C-7. The *meta*-coupled protons appearing at δ 7.2(1H) and 6.9(1H) in the ^1H NMR spectrum of **3** in $CDCl_3$ must be from the B-ring of the flavonoid moiety. The B-ring protons of 5,5'-dihydroxy-3,6,7,3',4'-pentamethoxyflavone (**4**) appeared as a 2H-singlet in ^1H NMR spectra of **4** taken in either $CDCl_3$ or acetone-d_6. Monomethyl **3** was found to be different (depressed mp) from **1**, indicating an oxygen substitution in the flavonoid B-ring for **3** different from that for **4**. These data show that **3** has an unusual oxygen substitution in the B-ring. One of the *meta*-coupled protons experiences a long-range coupling effect from an *ortho*-methoxy group. On the basis of the above information, **3** is characterized as 5,5'-dihydroxy-6,7,2',3'-tetramethoxyflavone.

From the bud exudate of *G. fosbergii* [1], five flavonoids were identified: 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone (**1**), 5,3',5'-trihydroxy-3,6,7,4'-tetramethoxyflavone (**2**), 5,4'-dihydroxy-3,6,7,8-tetramethoxyflavone (**5**) and two other flavonoids in small amounts. One of the minor flavonoids was shown to be identical with **3**.



1 $R^4 = R^5 = R^6 = OMe$; $R^1 = R^2 = R^3 = H$
2 $R^1 = R^5 = OMe$; $R^4 = R^6 = OH$; $R^2 = R^3 = H$
3 $R^3 = R^4 = OMe$; $R^6 = OH$; $R^1 = R^2 = R^5 = H$
4 $R^1 = R^4 = R^5 = OMe$; $R^6 = OH$; $R^2 = R^3 = H$
5 $R^1 = R^2 = OMe$; $R^5 = OH$; $R^3 = R^4 = R^6 = H$

EXPERIMENTAL

The bud exudates were collected from the *Gardenia* species growing in Batticaloa, Sri Lanka. Plant material was

*To whom correspondence should be addressed.

verified by Dr. D. Thiruvengadam (Mauritius). Mps are uncorr. The identities of all known compounds were confirmed by direct comparison with authentic samples (mmp, co-TLC and IR).

Extraction and fractionation of the bud exudate of G. cramerii. The bud exudate (37 g) was extracted with hot $CHCl_3$ in a Soxhlet apparatus for 12 hr. The solvent was removed under red. pres. to give a yellow gum. This extract (29 g) was partitioned between Et_2O and 10% aq. Na_2CO_3. The usual work-up gave acidic (20 g) and non-acidic (3.5 g) fractions. The acidic fraction in CH_2Cl_2 was washed with 10% aq. $Na_2B_4O_7$ soln and the organic layer on evapn gave a yellow gum (0.5 g). The Na_2CO_3-insoluble and $Na_2B_4O_7$-insoluble fractions were separated into their constituent pigments as described below.

The Na_2CO_3-insoluble fraction (200 mg) was separated by prep. TLC (Si gel) using $CHCl_3$–MeOH (9:1) into three pigments. The least polar pigment (58 mg) had mp 195–197°. It showed an orange-red colour with Mg–conc HCl and gave a green colour with neutral $FeCl_3$. It was identified as **1**. The most polar compound was found to have a mp 176–178° and was identical with **2**. The compound with intermediate polarity was re-purified by prep. TLC to give a light yellow crystalline solid (22 mg), **3**, mp 218° (from petrol–MeOH). It gave a red colour with Mg–conc HCl, olive green colour with $FeCl_3$ and a greenish blue colour with Gibbs reagent. IR ν_{max} 3360, 2910, 1660–1650, 1600, 1490–1450, 1420, 1360, 1280, 1250, 1200, 1100, 1010 and 825 cm^{-1}. UV λ_{max}^{MeOH} (log ϵ) 238 (4.11), 277 (4.00), and 330 (4.12); NaOAc 278 (4.19), 331 (4.30); H_3BO_3–NaOAc 278 (4.19), 331 (4.30); $AlCl_3$ 282 (3.92), 298 (3.92), 354 (4.05); $AlCl_3$–HCl 287 (3.92), 332 (3.90), 352 (4.05); ^1H NMR δ ($CDCl_3$) 3.82 (3H, *s*, –OMe), 3.93 (3H, *s*, –OMe), 3.96 (3H, *s*, –OMe), 4.0 (3H, *s*, –OMe), 6.53 (1H, *s*, 3–H), 6.6 (1H, *s*, 8–H), 6.9 (1H, *d*, J = 2 Hz, 4′–H) and 7.2 (1H, *d*, J = 2 Hz, 6′–H); δ (acetone-d_6) 3.8 (3H, *s*, –OMe), 3.9 (3H, *s*, –OMe), 4.0 (6H, *s*, –OMe), 6.75 (1H, *s*, 3–H), 6.85 (1H, *s*, 8-H) and 7.2 (2H, *s*, 4′–H and 6′–H); δ (DMSO-d_6) 3.75 (6H, *s*, –OMe), 3.9 (6H, *s*, –OMe), 6.92 (1H, *s*, 3–H), 6.96 (1H, *s*, 8–H), 7.2 (2H, *s*, 4′–H and 6′–H). MS *m/z* 374 (M^+ 100%), 359, 345, 331, 197, 181 and 153. M^+ 374.101 high-resolution MS, calc. for $C_{19}H_{18}O_8$, 374.102.

Methylation of **3**. Diazomethane methylation gave monomethyl **3**, mp 189–191°, which was found to be different from **1** since it gave a depressed mmp.

Extraction and fractionation of the bud exudate of G. fosbergii. The bud exudate (65 g) was extracted with $CHCl_3$ in a Soxhlet apparatus for 12 hr. The $CHCl_3$ extract on evapn of the solvent yielded a yellow gum (26 g) which was partitioned with Na_2CO_3 (10%) and Et_2O. Usual work-up gave the acidic (18 g) and non-acidic (5 g) fractions. The Na_2CO_3-insoluble fraction was re-extracted with EtOH [1]. The portion which dissolved was again extracted with CH_2Cl_2. The CH_2Cl_2-soluble fraction was chromatographed over Si gel and the column eluted with CH_2Cl_2–MeOH (99:1) giving a mixture of five flavonoids which were separated by prep. TLC. 200 mg of the mixture gave **1** (25 mg), mp 203–204°; **2** (90 mg), mp 176–178°; **5** (20 mg), mp 223–225°, **3** (15 mg) and another flavone (5 mg) mp 227–229°.

Acknowledgements—We thank Professor S. Balasubramaniam and Dr. D. Thiruvengadam for collecting and identifying the bud exudates and Dr. T. Nakanishi for providing mass spectral data. A research grant from the National Science Council of Sri Lanka is gratefully acknowledged.

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