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APIGENIN 7-GLUCOSIDE AND ITS 2″- AND 6″-ACETATES FROM LIGULATE FLOWERS OF *MATRICARIA CHAMOMILLA*

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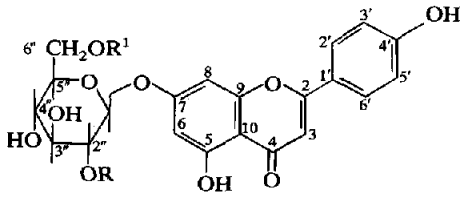
Abstract—Dried ligulate flowers of *Matricaria chamomilla* contain 7–9% glucosides of apigenin and 0.3–0.5% free apigenin. Glucosides were identified as apigenin 7-glucoside and a 1:3 mixture of the 2″- and 6″-acetates, as determined by ^{13}C NMR analysis.

INTRODUCTION

Among the various flavonoids isolated and identified in flowers of *Matricaria chamomilla* [1], apigenin (5,7,4′-trihydroxyflavone) and its glucosides play an important role, due to their pharmacological properties [2, 3]. We have now further investigated the apigenin glucosides present in the flowers.

RESULTS AND DISCUSSION

Dried tubular flowers of *Matricaria chamomilla* L. contain 0.2–0.3% of apigenin and its glucosides as a mixture with other flavones, from which separation is quite difficult. By contrast, dried ligulate flowers contain 7–9% of apigenin glucosides and 0.3–0.5% of the free aglucone. Due to the relatively high content of both free and bound apigenin, we carefully investigated the chemical constitution of the three glucosides **1–3**. **1** was readily recognized as apigenin 7-*O*-β-glucoside by comparison with authentic material. **2** and **3** were identical in their chromatographic properties and were inseparable. The IR spectrum of the mixture showed a band at 1715 cm^{-1} indicating that an ester moiety was present in the compounds. Alkaline hydrolysis afforded **1** thus confirming that **2** and **3** were esters of **1**. Furthermore, acetylation of the mixture in acetic anhydride–pyridine (100°, 3 hr) afforded a peracetate which was indistinguishable from the peracetate obtained from **1**. Interestingly, the permethyl derivative of the mixture sowed, as the only appreciable difference, in its ^{1}H NMR spectrum a peak at 2.05 ppm (*ca* 3H) and some slight modification in the δ methoxyl region. The IR of the permethylated mixture showed a band at 1740 cm^{-1}, strongly suggesting that it was a mixture of indistinguishable acetates at the glucosidic moiety. In order to confirm this and to clarify the site of binding of the acetyl group(s), ^{13}C NMR of **1** and **2**+**3** were recorded in DMSO-d_6 [4]. On the basis of assignment of shifts in flavonoid



1 R = R′ = H
2 R = H; R′ = Ac
3 R = Ac; R′ = H

Table 1. ^{13}C NMR of apigenin glucosides

	1	**2+3**
C-2	164.0	164.0
C-3	102.8	102.9
C-4	181.6	181.6
C-5	161.0	161.1
C-6	99.7	99.4
C-7	162.6	162.4
C-8	94.6	94.6
C-9	156.6	156.6
C-10	105.1	105.2
C-1′	120.7	120.7
C-2′	128.3	128.3
C-3′	115.7	115.8
C-4′	160.8	160.9
C-5′	115.7	115.8
C-6′	128.3	128.3
—C=O (acetyl)	—	169.9
—Me (acetyl)	—	20.5

	1	**2**	**3**
C-1″	99.3	99.4	98.3
C-2″	73.0	72.9	76.0
C-3″	77.0	76.6	74.2
C-4″	69.4	69.7	69.7
C-5″	76.3	73.7	76.3
C-6″	60.5	62.9	59.8

glucosides [5], we assigned the resonances to carbon atoms as described in Table 1.

From these data it is quite clear that the two spectra markedly differ in the resonances of the glucose carbons, the main difference being the signal at 169.9 ppm, due to the carbonyl of acetate(s). Moreover, qualitative evaluation of the pattern of resonances of glucose carbons in the spectrum of **2+3** clearly indicates it to be a mixture of at least two acetates. A careful examination of this portion of the spectrum indicates a downfield shift of C-6 (from 60.5 to 62.9 ppm) and an upfield shift of C-5 (from 76.3 to 73.7 ppm) thus indicating a C-6 acetyl in **2+3**. Furthermore, a signal at 59.8 ppm reveals that a C-6 hydroxyl is still present and we suggest that it can be assigned to C-6 of an acetyl glucoside present in mixture with the previously observed C-6 acetylate. Furthermore, an upfield shift of 1.0 ppm of C-1 (from 99.3 to 98.3 ppm) is accompanied by a signal at 76.0 ppm, which can be attributed to C-2 with a downfield shift of 3.0 ppm (from 73.0 of the unacetylated compound). Another signal at 74.2 ppm is assigned to C-3 with an upfield shift of 2.8 ppm from 77.0. From these assignments the structure of the 2″-acetate is proposed for the minor component present in the mixture. Furthermore, from the relative integrations of the assigned signals we can say that the 2″- and 6″-acetates are present in the approximate ratio of 1:3.

EXPERIMENTAL

Extraction and isolation. Dried ligulate flowers of *Matricaria chamomilla* (50 g; from Bulgaria) were continuously extracted with EtOH–H_2O (3:7) for 8 hr. After evapn to dryness, Et_2O (500 ml) was added and the mixture stirred (24 hr) and then filtered, washing the yellow solid with 3× 50 ml Et_2O. The yellow residue (8 g) was purified by column chromatography (700 g, Si gel Merck 60, 70–230 mesh ASTM) and elution performed with mixtures of $CHCl_3$–MeOH (1–30% of MeOH). Apigenin (0.18 g), **2+3** (0.37 g) and **1** (3.31 g) were obtained (TLC analysis on Si 60 F_{254} Merck plates, $CHCl_3$–MeOH, 3:2, exposition to I_2 or NH_3 vapours: apigenin R_f 0.78 (**2+3**) R_f 0.75, (**1**) R_f 0.64). Apigenin was crystallized from EtOH–EtOc, mp 345–350° (lit. 344–347°) [6]. (Found: C, 66.28; H, 3.62. $C_{15}H_{10}O_5$ requires: C, 66.67; H, 3.73%.) **1** was crystallized from MeOH, mp 180–182° (lit. 178–180°) [7]. ($C_{21}H_{20}O_{10}·H_2O$ requires: C, 56.00; H, 4.92. Found: C, 56.12; H, 4.64%.) **2+3** was a single spot in TLC, mp 250–252° (from MeOH); IRν max cm^{-1}: 3600, 1715; ^1H NMR (in DMSO-d_6, ppm from TMS): δ 2.05 (*s*, 3H), 3.35 (*m*), 3.95 (*m*), 4.15 (*m*), 4.35 (*m*), 5.15 (*m*), 6.50 (*m*, 1H), 7.00 (*d*, J = 9 Hz, 2H), 8.00 (*d*, J = 9 Hz, 2H). (Found: C, 56.27; H, 4.83%).

Permethylation of **1**. A solution of **1** (0.1 g) in dry DMF (15 ml) was added to a mixture of NaH (55% in oil) (0.05 g), washed twice with dry hexane, in dry DMF (15 ml). After 0.5 hr, an excess of MeI (0.5 ml) was added and the mixture stirred for additional 2 hr. The mixture was then poured into cold H_2O and after conventional work-up, the residue was purified by column chromatography; fractions eluted with $CHCl_3$–MeOH (99:1) were collected and the permethyl ether crystallized from MeOH; mp 198–199°. (Found: C, 62.60; H, 6.16. $C_{27}H_{32}O_{10}$ requires: C, 62.79; H, 6.24%). ^1H NMR (in $CDCl_3$): δ 3.30 (*m*, 2H), 3.41 (*s*, 3H), 3.60 (*s*, 3H), 3.70 (*s*, 6H), 3.87 (*s*, 3H), 3.96 (*s*, 3H), 5.00 (*m*, 1H), 6.55 (*m*, 2H), 6.75 (*m*, 1H), 6.92 (*m*, 1H), 7.00 (*d*, J = 9 Hz, 2H), 7.85 (*d*, J = 9 Hz, 2H).

1 was acetylated with Ac_2O/Py for 3 hr at 100° to yield the acetate; mp 225–226° (from 96% EtOH); ^1H NMR (in $CDCl_3$): δ 2.10 (*s*, 3H), 2.35 (*s*, 3H), 2.47 (*s*, 3H), 4.25 (*s*, 3H), 5.32 (*s*, 4H), 6.60 (*s*, 1H), 6.74 (*d*, J = 2.5 Hz, 1H), 7.05 (*d*, J = 2.5 Hz, 1H), 7.30 (*d*, J = 9 Hz, 2H), 7.90 (*d*, J = 9 Hz, 2H).

Permethylation of **2+3**. This was performed as described above for **1**. Mp 187–188° (from MeOH). (Found: C, 63.00; H, 5.92%). IR cm^{-1}: 1740; ^1H NMR (in $CDCl_3$): δ 2.05 (*s*, 3H) and all the other resonances unchanged (see **1**).

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REFERENCES

1. Tyihak, E., Sarkany-Kiss, I. and Verszar-Petri, G. (1962) *Pharmazie* **5,** 301.
2. Hava, M. and Janku, J. (1958) *J. Physiol.* **50,** 309.
3. Sörm, F., Cekan, Z. and Raskova, H. (1952) *Chem. Listy* **46,** 308.
4. Chari, V. M., Jordan, M., Wagner, H. and Thies, P. W. (1977) *Phytochemistry* **16,** 110.
5. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34,** 1389.
6. Power, F. B. and Browning, H. (1914) *J. Chem. Soc.* **105,** 2280.
7. Nordström, C. G. and Swain, T. (1953) *Chem. Ind. (London)* 85.