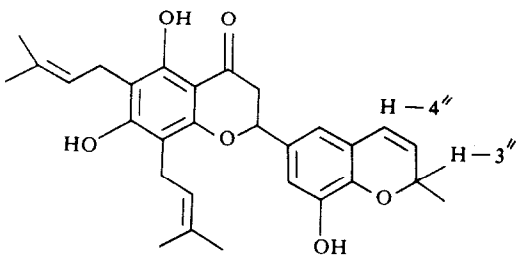

1

12.31 (1H) and the multiplet at δ 2.90 (2H) are characterisic for a chelated-OH and H-3, respectively [2].

The mass spectrum gave the molecular formula $C_{30}H_{34}O_6$ for amorinin (1). The RDA fragment *m/z* (rel. int.) at 288 (3) represents two hydroxyl and two prenyl groups on ring A. This fragment further stabilizes by the loss of $-C_3H_7$, $-C_4H_7$ and $-C_3H_7$–C_4H_8 at *m/z* 245 (11), 233 (21) and 189 (49) [3, 4]. The 45-nm bathochromic shift of the 300-nm band in the UV spectrum upon addition of NaOAc suggests the non-chelated hydroxyl to be at C-7 [5]; hence the prenyl groups can be located to C-6 and C-8, respectively.

The RDA fragment at *m/z* 202 (23) and the ion at *m/z* 187 (55) arising from it by the loss of a methyl group indicate that ring B has one dimethylchromene and one hydroxyl group. The *meta*-coupled doublets at δ 6.89 (1H, $J = 2.2$ Hz, H-2′) and 6.62 (1H, $J = 2.0$ Hz, H-6′) (Δ H-6′ and H-2′ 0.27 ppm) indicate the attachment of the dimethylchromene ring system at the 4′, 5′-positions and the hydroxyl group at C-3′, as in structure 1. For the isolated compound the trivial name amorinin (1) is proposed.

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APIGENIN 7-GLUCOSIDE DIACETATES IN LIGULATE FLOWERS OF *MATRICARIA CHAMOMILLA*

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Key Word Index—*Matricaria chamomilla*; Compositae; ligulate flowers; apigenin glucosides; apigenin 7-*O*-β-glucoside diacetates; ^{13}C NMR.

Abstract—From ligulate flowers of *Matricaria chamomilla* was isolated a mixture of apigenin 7-*O*-β-glucoside diacetates, which was shown to be based on (2″, 3″)- and (3″, 4″)-diacetates.

INTRODUCTION

Apigenin (5, 7, 4′-trihydroxyflavone) and its glucosides are the main flavonoids of *Matricaria chamomilla* L. [1]. Tyihak *et al.* have already shown by PC that a few unspecified apigenin glucosides are present in the flower of the above plant [2]. Apart from the most abundant 7-*O*-β-glucoside (1), also monoacetates, mainly 6″-acetate (2), are present in the flowers [3, 4]. We now present evidence that a mixture of diacetates, 3 and 4, can be isolated from the same source.

RESULTS AND DISCUSSION

During our work of standardization of active components of *Matricaria chamomilla* L. [1, 5, 6], it happened that from ligulate flowers of some plants, collected either in particular places or times of the year, it was sometimes possible to isolate by CC a fraction of polarity similar to the monoacetate (2). This compound was apparently also homogeneous when analysed by reverse phase HPLC with the system of elution described for 1 and 2 [1]. In fact the chromatogram exhibited a single peak with a retention

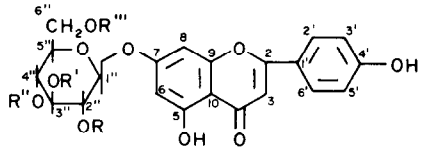
Table 1. ^{13}C NMR of apigenin glucoside diacetates 3 and 4 (in DMSO-d_6)

			3	4
C-2	164.0	C-1″	99.4	99.4
C-3	102.9	C-2″	74.4	72.9
C-4	181.6	C-3″	77.4	77.4
C-5	161.1	C-4″	67.2	70.9
C-6	99.4	C-5″	76.6	73.6
C-7	162.4	C-6″	60.2	60.2
C-8	94.6			
C-9	156.6			
C-10	105.2			
C-1′	120.7			
C-2′	128.3			
C-3′	115.8			
C-6′	128.3			
–C=O (acetyl)	169.6, 169.3			
–Me (acetyl)	20.5			

time of 8.2 min, whereas **1** was eluted in 3 min and **2** in 12.3 min. The IR spectrum of the above fraction was substantially identical with **2**, the most significant band being at 1740 cm^{-1}. Furthermore, alkaline hydrolysis of the fraction quantitatively yielded **1**. From the ^1H NMR spectrum no additional information was obtained, since the spectrum was identical to the one exhibited by **2** apart from the intensity of the signal at 2.05 ppm, which was consistent with six hydrogens. From these preliminary data, a diacetate of **1** was most likely and MS confirmed this hypothesis. In fact, in the mass spectrum a molecular ion at *m/z* 516 clearly indicated that the fraction isolated was a diacetate. On the other hand, Kunde and Isaac had already found a diacetate of **1** as an impurity of their 6″-acetate (**2**), since in the mass spectrum of **2** a peak at *m/z* 516 was detected [4]. From our work-up of the ligulate flowers, we isolated enough material for structural elucidation by means of ^{13}C NMR. In fact, the ^{13}C NMR of our diacetate was run in DMSO-d_6 and the presence of two acetoxy groups was confirmed by two resonances at 169.3 and 169.6 ppm (Table 1). The most significant resonances, however, were in the glucosidic portion of the spectrum. A resonance at 60.2 ppm indicated that the primary alcohol at C-6″ was unsubstituted and the presence of six peaks in the range 67.2–77.4 ppm suggested a mixture of diacetates.

We propose that ligulate flowers of *Matricaria chamomilla* contain a mixture of (2″,3″) (**3**) and (3″,4″) (**4**) diacetates for the following reasons. We assign to **3** and **4** the resonances as shown in Table 1, starting from the well-established downfield shift of the carbon bearing the acetoxy group and upfield shift for the adjacent carbon. The situation of the anomeric carbon C-1″ is indicative for substitution at C-2″ and the ^{13}C NMR spectrum of **3**+**4** shows that the C-1″ resonance is almost unchanged, when compared with the spectrum of **1** [3]. We propose the structure of the 3″,4″-diacetate to **4**, since for this compound resonances as indicated in Table 1 can be assigned.

On the other hand, since hydrolysis of **3**+**4** affords **1** and C-6″ is unsubstituted, only two structures of 2″,4″- and 2″,3″-diacetates are possible for **3**. However, the C-1″ of a 2″,4″-diacetate should be affected to a large extent by the presence of an acetoxy group at C-2″. We therefore suggest that **3** is a 2″,3″-diacetate and that the resonances of carbons at positions 1″ and 2″ are not too different from the same values in **1**. Further, a rough estimation of the intensities of the signals indicates an almost equimolar amount of **3** and **4**. Finally, HPLC analysis of flowers from different sources has shown to us that the total amount of the diacetates is variable and in many samples almost not detectable.



1 R=R′=R″=R‴=H
2 R=R′=R″=H, R‴=Ac
3 R=R′=Ac, R″=R‴=H
4 R=R‴=H, R′=R″=Ac

EXPERIMENTAL

Extraction and isolation. Dried ligulate flowers of *Matricaria chamomilla* (50 g; from Egypt, 1980) were continuously extracted with either EtOH or MeOH–H_2O (3:7) for 8 hr. After evapn to dryness, Et_2O (500 ml) was added and the mixture stirred and then filtered, washing the yellow solid with 3 × 50 ml Et_2O. The residue (8 g) was purified by CC (700 g, Si gel, Merck 60, 70–230 mesh ASTM), eluting with mixtures of $CHCl_3$–MeOH (1–30% of MeOH). The fractions corresponding to **1** were collected (3 g) and chromatographed again on a column, eluting with $CHCl_3$–MeOH (9:1). The mixture of **3** and **4** was crystallized from MeOH, mp 156° (decomp.). ($C_{25}H_{24}O_{12}$ requires: C, 58.14, H, 4.65. Found: C, 58.32, H, 4.48%.) **3**+**4** were a single spot in TLC (on Si 60 F_{254} Merck plates, $CHCl_3$–MeOH, 3:2, exposure to I_2 vapours); IR ν_{max} cm^{-1}: 1740; ^1H NMR (in DMSO-d_6): 2.05 (*s*, 6H), 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) ppm from TMS. MS *m/z* 516 (M^+), 474 ($M^+ - 42$), 418, 270, 242, 187, 153.

HPLC analysis. A Perkin–Elmer Series 3 liquid chromatograph, microprocessor controlled pump module, equipped with a Rheodine injector, a Model 023 recorder and UV liquid chromatography analyser (Model 55B) as detector was used for the analyses. The column was a Perkin–Elmer 0.26 × 25 cm HC-ODS Sil-X reverse-phase column, the mobile phase was a concave exponential n. 2 (curve 2) gradient increasing in 25 min from 15 to 60% acetonitrile in water containing 2% HOAc. The flow rate was 1 ml/min, detection at 335 nm and the sample injected had a vol. of 5 μl. The peak-width parameter value of integrator was 5 and slope sensitivity 350. The retention times are quoted in the text.

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CHRYSOERIOL 7-(2″-*O*-β-D-ALLOPYRANOSYL)-β-D-GLUCOPYRANOSIDE FROM *SIDERITIS GRANDIFLORA*

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Key Word Index—*Sideritis grandiflora*; Labiatae; ^{13}C NMR; chrysoeriol 7-(2″-*O*-β-D-allopyranosyl-β-D-glucopyranoside).

Abstract—The aerial parts of *Sideritis grandiflora* yielded a new flavone glycoside, identified as chrysoeriol 7-(2″-allosylglucoside).

As part of our programme on the study of the chemical components of *Sideritis* species we had occasion to investigate the flavonoid fraction of *S. grandiflora*. This species is endemic in the south-west of the Iberian peninsula. Methanol extraction of the dried aerial parts of this plant yielded a mixture of flavonoid glycosides (0.67%), the main component of which was isolated as the acetate by prep. TLC of the total acetylated mixture as well as that of the glycosidic fraction itself. Acid hydrolyses of this glycoside (SG-1) yielded chrysoeriol as the aglycone and glucose and a second hexose were detected as the sugars. Elemental analysis ($C_{46}H_{50}O_{25}$) and mass spectrometry of the peracetate (M^+ m/z 1002) also confirmed the presence of two hexose residues in the molecule.

Methylation of the natural product SG-1 with dimethyl sulphate–potassium carbonate in acetone and subsequent hydrolysis yielded a compound whose properties were the same as those reported for 7-hydroxy-5, 3′, 4′-trimethoxyflavone [1]. This thus established that the chrysoeriol is linked at position 7 with a disaccharide moiety. The ^1H NMR spectrum of the chrysoeriol bioside in deuteropyridine at 300 MHz showed two doublets for the anomeric protons at δ 5.75 ($J = 7.1$ Hz) and at 5.88 ($J = 8.2$ Hz), respectively. The values of the coupling constant indicate that the two hexose moieties are linked in a 1, 2-*trans* diequatorial orientation and are in the pyranose forms. The lack of resonances in the region δ 2.0 ± 0.5 indicated the absence of acetyl groups. The nature of the second hexose residue as well as the position of the interglycosidic linkage in the natural product was determined by ^{13}C NMR spectroscopy. The signal at δ 67.3 in the ^{13}C NMR spectrum of the compound for an oxymethine carbon atom in a β-hexopyranosyl residue, was indicative of an unsubstituted C-4 in a β-allopyranosyl moiety [2]. Acidic hydrolysis of the glycoside and subsequent GC analysis of the sugars confirmed that the second hexose unit was indeed allopyranose. The R_f values of allose and glucose, on TLC and PC, in most solvent systems are very similar and the former can very easily be mistaken for the latter which is of more widespread occurrence. The signal at δ 82.3 must be clearly that of the sugar carbon atom bearing the second glycosyl moiety. This value is more downfield than would be expected if glucose were the terminal sugar and linked to allose in the positions C-2, C-3 or C-6. The chemical shift values for these carbon atoms are δ 70.6, 71.3 and 61.4, respectively [3]. Alternatively, if allose is the terminal sugar in the