

## FLAVONES FROM *EUPATORIUM LEUCOLEPIS*\*

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**Key Word Index**—*Eupatorium leucolepis*; Compositae; Eupatorieae; flavones; 3',4'-methylenedioxy-5,6,7,8-tetramethoxyflavone; 3',4'-methylenedioxy-5,6,7,8,5'-pentamethoxyflavone; nobiletin; 3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone; 4'-hydroxy-5,6,7,8,3',5'-hexamethoxyflavone; 4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone.

**Abstract**—Chloroform extraction of *Eupatorium leucolepis* gave the new flavones 3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone, 4'-hydroxy-5,6,7,8,3',5'-hexamethoxyflavone and 4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone. 3',4'-Methylenedioxy-5,6,7,8-tetramethoxyflavone, 3',4'-methylenedioxy-5,6,7,8,5'-pentamethoxyflavone and nobiletin were also isolated.

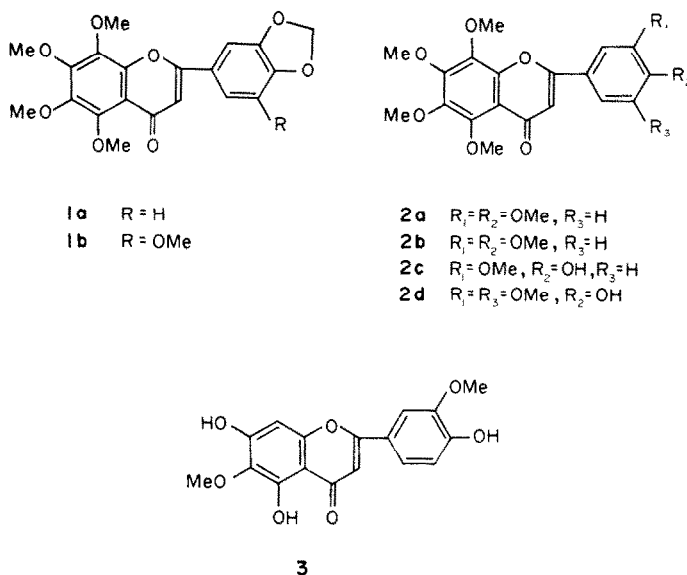
### INTRODUCTION

In continuation of our study of *Eupatorium* species which characteristically elaborate sesquiterpene lactones with cytotoxic or antitumor activity [1] we have investigated *Eupatorium leucolepis* which is found in the coastal plain of the south-eastern United States [2–4]. Although no lactones were isolated we found small amounts of three new flavones **2b**, **2c** and **2d** as well as larger quantities of the previously known flavones **1a**, **1b**, **2a** and **3**.

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### RESULTS AND DISCUSSION

Compound **2b**,  $C_{21}H_{22}O_9$  (high resolution mass spectrum), was a monohydroxyhexamethoxyflavone whose oxygen functions were distributed over C-5, C-6, C-7, C-8, C-3', C-4' and C-5' of the ring system ( $^1H$  NMR and mass spectrum). The lone hydroxyl was not on C-5 (no chelated hydroxyl signal in the  $^1H$  NMR spectrum, unchanged UV spectrum on addition of aluminium chloride) or C-4' (bathochromic shift of band I by only 18 nm on addition of sodium methoxide), while the mass spectral fragmentation (Scheme 1) indicated [5] that it was located in ring B and not in ring A. Hence the hydroxyl group was attached to C-3' and the substance was 3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone. In accordance with this deduc-



Scheme 1. Selected mass spectral fragments of **2b**.

tion, a large benzene-induced upfield shift was observed for only one of the six methoxyl signals [6].

Compound **2c**,  $C_{20}H_{20}O_8$  (high resolution mass spectrum), was a monohydroxypentamethoxyflavone whose oxygen functions were located on the C-5, C-6, C-7, C-8, C-3' and C-4' positions [ $^1H$  NMR, mass spectrum (see Scheme 2)]. The hydroxyl group was not on C-5 (no chelated hydroxyl signal in the  $^1H$  NMR spectrum), but on C-4' (71 nm bathochromic shift of band I on addition of sodium methoxide, 69 nm shift on addition of sodium acetate). Hence the substance was 4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone; again only one of the methoxy signals exhibited a large benzene-induced upfield shift.

Compound **2d**,  $C_{21}H_{22}O_9$  (high resolution mass spectrum), was another monohydroxy-hexamethoxyflavone whose oxygen functions were located on C-5, C-6, C-7, C-8, C-3', C-4' and C-5' ( $^1H$  NMR spectrum). The most obvious feature on the  $^1H$  NMR spectrum, apart from the absence of a chelated hydroxyl, was the coincidence of the H-2' and H-6' resonances. Hence ring B was symmetrically substituted. Since the free hydroxyl group was located in ring B (mass spectrum, see Scheme 3) the substance was 4'-hydroxy-5,6,7,8,3',5'-hexamethoxyflavone. This deduction was supported by the UV spectrum which was unchanged on addition of aluminium chloride but exhibited 81 and 79 nm bathochromic shifts of band I on addition of sodium methoxide and sodium acetate, respectively, and by the observation that two of the

six methoxyl signals exhibited large benzene-induced upfield shifts.

Nearly 30 taxa included in *Eupatorium sensu stricto* [7] have so far been investigated chemically [1]\*. Only a few, e.g. *E. capillifolium* [9], *compositifolium* [9], *pinnatifidum* [9], *leptophyllum* [9], *petaloideum* [10]†, *album* [11] and *pilosum* [1, 12] to which must now be added *E. leucolepis*, do not seem to elaborate sesquiterpene lactones in detectable amounts. Whether this possesses taxonomic significance is not known.

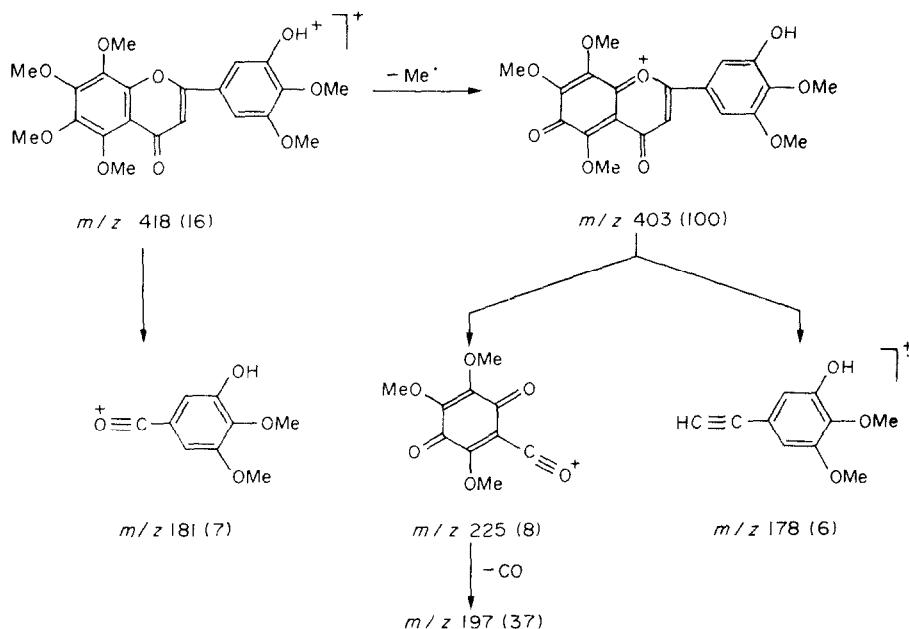
#### EXPERIMENTAL

Above-ground dried material of *E. leucolepis* T. & G. (2.3 kg), collected by Dr. R. K. Godfrey on Aug. 4, 1968, along Florida Route 67 four miles N. of Carabelle, Franklin Co., Florida (RK667978 on deposit in Herbarium of Florida State University), was extracted with  $CHCl_3$  and worked-up in the usual manner [13]. The crude gum (47 g) obtained was preadsorbed on 75 g silicic acid (Mallinckrodt 100 mesh) and chromatographed over 0.75 kg silicic acid packed in  $C_6H_6$ . Fractions were collected as follows: fractions 1–10 ( $C_6H_6$ , 41.), 11–20 ( $C_6H_6-CHCl_3$ , 1:1, 41.), 21–28 ( $CHCl_3$ , 31.), 29–35 ( $CHCl_3-MeOH$ , 99:1, 31.), 36–40 ( $CHCl_3-MeOH$ , 49:1, 21.), 41–47 ( $CHCl_3-MeOH$ , 19:1, 31.), 48–52 ( $CHCl_3-MeOH$ , 9:1, 21.) and 53–57 ( $CHCl_3-MeOH$ , 4:1, 21.).

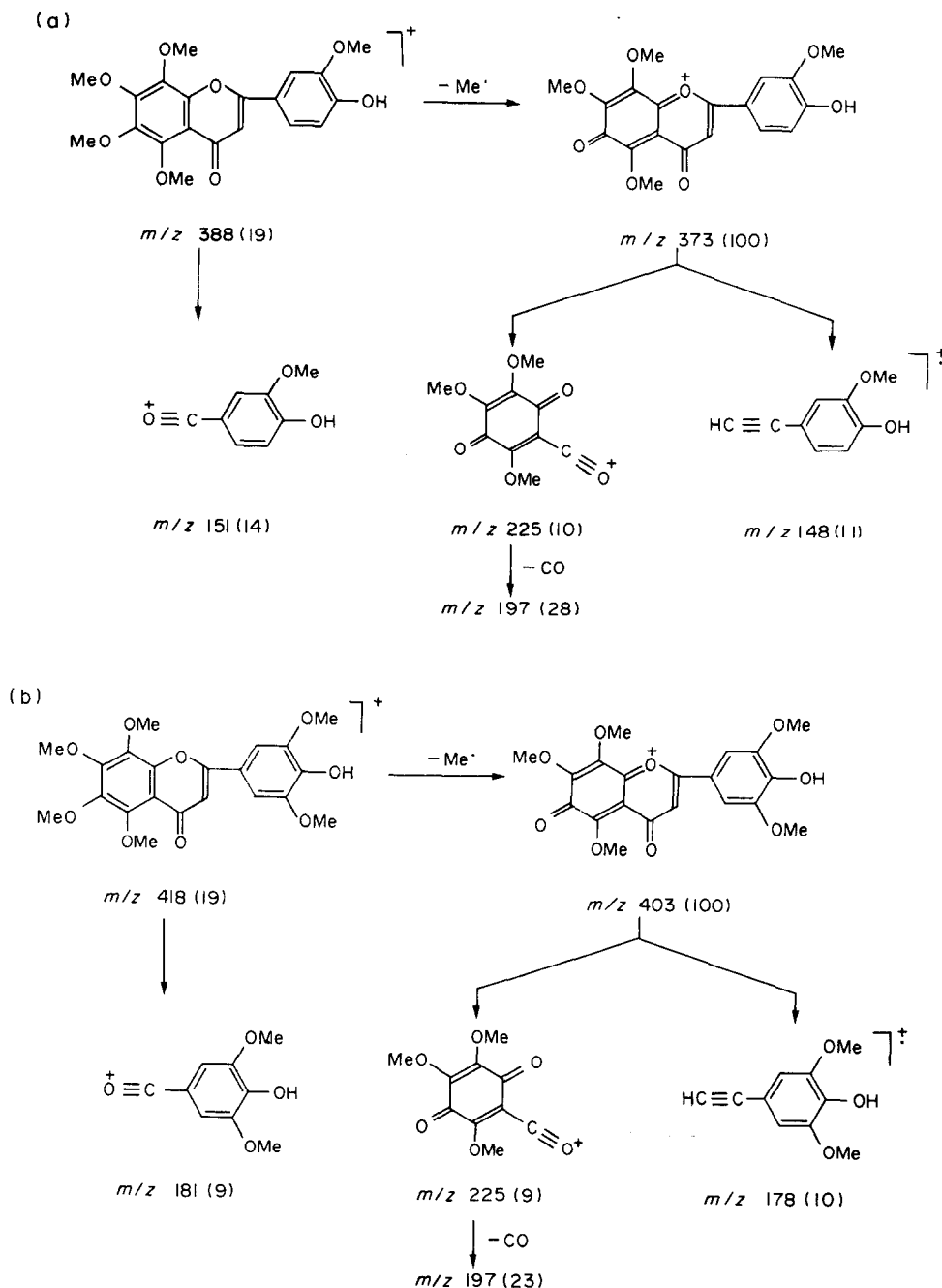
Fractions 33 and 34 were combined (10.2 g) and triturated with  $Et_2O$  to afford a crystalline mass. Purification of a 150 mg portion by TLC (hexane- $EtOAc$ , 3:1, once and hexane- $EtOAc$ , 1:1, twice) gave three flavonoids. The least polar substance, 3',4'-methylenedioxy-5,6,7,8-tetramethoxyflavone (**1a**) crystallized from  $CHCl_3-MeOH$  (35 mg), mp 169–170° (lit. 160° [14], 171–172° [15]) and was identified as linderflavone B [16] by NMR and MS. 3',4'-Methylenedioxy-5,6,7,8,5'-pentamethoxyflavone (**1b**) crystallized from  $CHCl_3-MeOH$  (40 mg), mp 187–188° [14], 190–191.5° [17] and was identified by NMR and comparison with

\*We include among this number several hybrid biotypes [2, 3] such as *E. anomalum* [8], *mohrii* [8] and *pinnatifidum* [9].

†This was originally misidentified as *E. album*.



Scheme 2. Selected mass spectral fragments of **2c**.

Scheme 3. Selected mass spectral fragments of **2d**.

an authentic sample. The most polar flavone **2a** (nobiletin) mp 133–134° (lit. 136.5–137.5° [18]) (20 mg) was also identified by NMR and comparison with an authentic sample.

Purification of fractions 36–37 (200 mg) by TLC (CHCl<sub>3</sub>–MeOH–EtOAc, 18:1:1) gave a flavone mixture. Further purification by TLC (C<sub>6</sub>H<sub>6</sub>–EtOAc, 1:1, twice) afforded three fractions, each of which were repurified by TLC (C<sub>6</sub>H<sub>6</sub>–EtOAc, 1:1, twice) to give three new flavones. The least polar substance, 3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone (**2b**), crystallized from CHCl<sub>3</sub>–MeOH (5 mg), mp 191–193°, <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.13 (*d*, 1.5 Hz, H-2', H-6'), 6.61 (H-3), 4.10, 4.02, 3.99, 3.95, 3.95, 3.95, (OMe); (C<sub>6</sub>D<sub>6</sub>)  $\delta$

7.05 (*d*, *J* = 1.5 Hz, H-2', H-6'), 6.63 (H-3), 4.03, 3.73, 3.72, 3.63, 3.51, 3.24 (OMe) UV  $\lambda_{\max}$  nm: MeOH 272, 318, 332<sub>sh</sub>; (MeOH–AlCl<sub>3</sub>) unchanged; (MeOH–NaOMe) 236, 254, 336. [Calc. for C<sub>21</sub>H<sub>22</sub>O<sub>9</sub>: MW, 418.1264. Found: MW (MS): 418.1235 (15.9%).] Other significant peaks in the low resolution MS were at *m/z* (rel. int.) 403 (100), 225 (8), 195 (37), 181 (7) and 178 (6).

The second new flavone 4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone (**2c**), could not be induced to crystallize (6 mg) NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.52 (*dd*, *J* = 9, 1.5 Hz, H-6'), 7.39 (*d*, *J* = 1.5 Hz, H-2'), 7.04 (*d*, *J* = 9 Hz, H-5'), 6.60 (H-3), 4.10, 4.02, 3.99, 3.96, 3.96 (OMe); (C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.33 (*dd*,

$J = 9$ , 1.5 Hz, H-6'), 7.07 ( $d J = 1.5$  Hz, H-2'), 6.92 ( $d J = 9$  Hz, H-5'), 6.67 (H-3), 4.04, 3.77, 3.74, 3.67, 3.16 (OMe); UV  $\lambda_{\max}$  nm: (MeOH) 248, 268, 272, 340; (MeOH-AlCl<sub>3</sub>) unchanged; (MeOH-AlCl<sub>3</sub>-HCl) unchanged; (MeOH-NaOMe) 239 sh, 260, 401; (MeOH-NaOAc) 258, 272, 398. [Calc. for C<sub>20</sub>H<sub>20</sub>O<sub>8</sub>: MW, 388.1158. Found: MW (MS), 388.1160 (19.4%).]. Other significant peaks in the low resolution MS were at  $m/z$  (rel. int.) 373 (100), 225 (10), 197 (28), 151 (14), 148 (11).

The most polar new flavone, 4'-hydroxy-5,6,7,8,3',5'-hexamethoxyflavone (**2d**) also could not be induced to crystallize (4 mg). <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.20 (2p, H-2', 6'), 6.32 (H-3, 4.11, 4.03, 4.00, 4.00, 3.96, 3.96 (OMe); (C<sub>6</sub>D<sub>6</sub>)  $\delta$  6.99 (H-2', H-6'), 6.71 (H-3), 4.07, 3.79, 3.75, 3.67, 3.30, 3.30 (OMe); UV  $\lambda_{\max}$  nm: (MeOH) 248sh, 270, 339; (MeOH-AlCl<sub>3</sub>) unchanged; (MeOH-AlCl<sub>3</sub>-HCl) unchanged; (MeOH-NaOMe) 238sh, 270sh, 316, 420; (MeOH-NaOAc) 248sh, 270, 336, 418. [Calc. for C<sub>21</sub>H<sub>22</sub>O<sub>9</sub>: MW, 418.1264. Found: MW (MS), 418.1265 (18.7%).] Other significant peaks in the low resolution MS were at  $m/z$  (rel. int.) 403 (100), 225 (9), 197 (23), 181 (9), 178 (10).

Fractions 42-44 (250 mg) were triturated with MeOH. The solid residue (70 mg) upon crystallization from Me<sub>2</sub>CO furnished 6,3'-dimethoxy-5,7,4'-trihydroxyflavone (jaceosidin, **3**), mp 218-219°, lit. 227-228° [19], 219-221° [20], which was identified by UV and NMR spectroscopy of its triacetate [19]. UV  $\lambda_{\max}$  nm: (MeOH) 349, 274; (MeOH-AlCl<sub>3</sub>) 389, 306, 281, 257; (MeOH-AlCl<sub>3</sub>) 376, 306, 284, 257; (MeOH-NaOMe) 416, 348, 256; (MeOH-NaOAc) 400, 311, 275; (MeOH-NaOMe-H<sub>3</sub>BO<sub>3</sub>) 349, 275; <sup>1</sup>H NMR of triacetate (mp 197-198°) (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.44 ( $d J = 9.2$  Hz, H-6'), 7.38 ( $d J = 2$  Hz, H-2') 7.29 (H-8), 7.16 ( $d J = 9$  Hz, H-5'), 6.59 (H-3), 3.91, 3.87 (OMe), 2.48, 2.39, 2.34 (Ac); (C<sub>6</sub>D<sub>6</sub>) H-6' and H-2' signal obscured by C<sub>6</sub>H<sub>6</sub> absorption,  $\delta$  6.44 (H-8, H-5', H-3), 3.74, 3.24 (OMe), 2.34, 1.89, 1.81 (Ac).

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