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BAHIFOLIN, A NEW SESQUITERPENE LACTONE, AND 5,7-DIHYDROXY-3,3',4',6-TETRAMETHOXYFLAVONE, A NEW FLAVONE, FROM *BAHIA OPPOSITIFOLIA*

WERNER HERZ, S V BHAT and HERSCHEL CRAWFORD
Department of Chemistry, The Florida State University, Tallahassee, Florida 32306, U S A

and

H WAGNER, G MAURER and L FARKAS*
Institut fur Pharmazeutische Arzneimittellehre der Universitat Munchen, 8 Munchen 2, Germany

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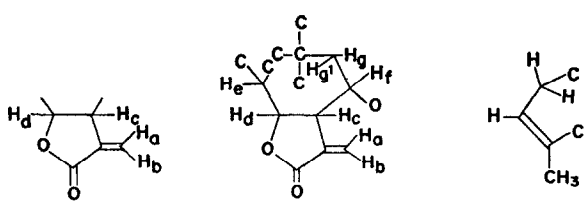
Abstract—Extraction of *Bahia oppositifolia* (Nutt) DC (Compositae) furnished a new sesquiterpene lactone bahifolin (I c) and the new flavone 5,7-dihydroxy-3,3',4',6-tetramethoxyflavone (III a) whose structure was confirmed by synthesis from jaceidin (III e) Extraction of *Bahia absinthifolia* Benth var *dealbata* (Gray) Gray gave the known sesquiterpene lactones bahia I (I a) and bahia II (I b)

IN THE course of our phytochemical studies in the tribe Heleniеae of Compositae we had occasion to investigate *Bahia oppositifolia* (Nutt) DC. Two crystalline, previously unknown compounds were isolated: A new sesquiterpene lactone bahifolin (I c) whose structure was established by correlation with bahia I (I a) and bahia II (I b),[1] newly isolated in the course of this work from *Bahia absinthifolia* Benth var. *dealbata* (Gray) Gray,[2] and a new flavone which was identified as 5,7-dihydroxy-3,3',4',6-tetramethoxyflavone (III a) and synthesized from jaceidin (III e)

Bahifolin (Ic), $C_{20}H_{20}O_6$, m p. 140–142°, $[\alpha]_D$ +14 3°, was a conjugated γ-lactone (IR bands at 1764 and 1662 cm^{-1}, strong UV end absorption) The NMR spectrum (Table 1) exhibited the typical two doublets due to H_a and H_b of partial structure A This was confirmed by spin decoupling experiments Irradiation of the H_c multiplet at 3·28 ppm collapsed doublets at 5 57 (H_a) and 6 29 (H_b) to singlets, a doublet of doublets at 4 61 (H_d) to a doublet and a triplet of doublets at 5 74 (H_f) to a triplet In turn, irradiation of H_f collapsed the multiplet of H_c to a broadened doublet and simplified the AB part centered at 2 14 ppm (H_g and H_g')—of an ABX system, thus allowing expansion of A to B.



* Permanent address Hungarian Academy of Sciences, Budapest

[1] A ROMO DE VIVAR and A ORTEGA, *Can J Chem.* **47**, 2849 (1969).
[2] W L ELLISON, *Rhodora* **66**, 67, 177, 281 (1964)

TABLE 1 NMR SPECTRUM OF BAHIFOLIN (90 mHz)[a]

H-1	[b]	
H-2	[b]	
H-3	5 55[c]	
H-5	[b]	
H-6	4 61dd	$J_{6,7} = 8\,5$, $J_{5,6} = 9\,5$
H-7	3 28 m	$J_{7,8} = 3\,9$, $J_{7,13a} = 3\,1$, $J_{7,13b} = 3\,6$
H-8	5 74 td	$J_{8,9} = 7\,7$[d]
H-9	[e]	
H-13a	5 57d	
H-13b	6 29d	
H-14a	2 75	$J_{14a,14b} = 5\,0$
H-14b	2 69[f]	
H-15	1 91d[g]	$J_{3,15} = 1\,5$
H-2′	7 96dd	$J_{2',4'} = 0\,6$, $J_{2',5'} \sim 1\,6$
H-4′	6 66dd	$J_{4',5'} = 2\,0$
H-5′	7 41 t	

[a] In deuteriochloroform solution using tetramethylsilane as internal standard Chemical shifts in parts per million, coupling constants in Hz Signals are denoted in the usual way d—doublet, t—triplet, m—multiplet Coupling constants by spin decoupling

[b] In four proton multiplet at 2 4 ppm

[c] Superimposed on H-13a

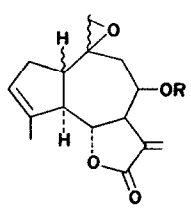
[d] Triplet separated by 7 7 Hz

[e] Only some lines of the AB part of an ABX system were visible at 2 38, 2 30, 2 22 and 2 14 ppm, and collapsed to broad lines at 2 37 and 2 20 on irradiation of H-8

[f] Broadened by long-range coupling to H-1 In (I a), (I b), and (II) the AB system of H-14a and H-14b has collapsed to a singlet

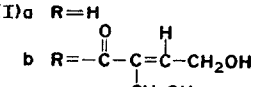
[g] Three protons

Further irradiation at 5·53 (vinyl proton superimposed on H_a) collapsed a narrowly-split vinyl methyl signal at 1 91 ppm to a singlet and simplified a complex four proton signal at 2·41 ppm thus indicating the presence of partial structure C These observations coupled with the presence of a two proton AB quartet near 2 7 ppm suggested that bahifolin possessed the carbon skeleton, oxygenation pattern and stereochemistry of bahia I (I a) and bahia II (I b), two sesquiterpene lactones previously[1] isolated from *Bahia pringlei* Greenm The isolation of small amounts of (I a) and (I b) from a collection of *Bahia absinthifolia* Benth. var *dealbata* (Gray) Gray permitted verification of this hypothesis Treatment of (I b) and bahifolin with methanolic potassium carbonate followed by acetylation of the neutral fraction yielded identical acetates (II)

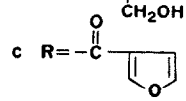


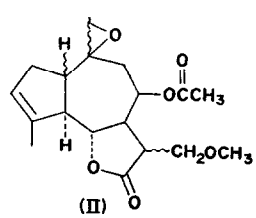
(I)a R=H

b R=



c R=





(II)

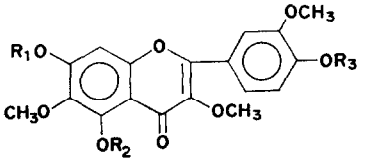
The presence of a 3-furoyl group, whose biogenetic relationship to the ester side chain of (I b) is obvious, was established spectroscopically. The high resolution mass spectrum of (I c) had its base peak at *m/e* 95·0134 ($C_5H_3O_2$) and also peaks at *m/e* 261·1134 ($C_{15}H_{16}O_4$) and 244·1104 ($C_{15}H_{16}O_3$). The IR spectrum had furan bands at 1572 and 878 cm^{-1}; the UV spectrum exhibited characteristic 3-furoyl absorption at 239 nm (ε 4380).[3]* In the NMR spectrum, (Table 1) H-5′ was identified as a triplet [4]†

Irradiation at a frequency corresponding to the signal at 7·41 (H-5′) collapsed the multiplets of H-2′ and H-4′ to doublets, in turn irradiation at 7 96 ppm (H-2′) collapsed the signals of H-5′ and H-4′ to doublets.

The strongly negative Cotton effect exhibited by bahifolin (λ_{max} 255 nm, θ −2200) is in accord[5] with the previously-deduced[1] *trans*-fusion of the lactone ring.

The second compound from *B oppositifolia* was a new flavone, $C_{19}H_{18}O_7$, m p 152–153° (or 158–160°, see Experimental), which had u.v. maxima at 349, 273 and 255 nm and contained four methoxyl (NMR spectrum) and two hydroxyl groups (formation of a diacetate) The distribution of functional groups in ring B was indicated by the NMR spectrum ($CDCl_3$) which displayed the typical AB system of H-5′ and H-6′ at 6·93 and 7·69 ppm. The signal of H-6′ was additionally split by coupling to H-2′ at 7·60 ppm. A singlet at 6 50 ppm could be attributed to either H-3 or H-8,[6,7] that C8- was unsubstituted was established by preparation of the dimethylether which was identical in all respects with quercetagetin hexamethyl ether (3,3′,4′,5,6,7-hexamethoxyflavone, III b).

Color reactions (positive ferric chloride test, yellow Mg–HCl and NaOH tests) suggested the presence of a 5-hydroxyl and the absence of a free 3-hydroxyl group. This was supported by the NMR spectrum (—OH band at 12·36 ppm) and the bathochromic shifts of bands I and II in the presence of $AlCl_3$.[8] That the second hydroxyl group was sited at C-7 was indicated by the bathochromic shift of band II (18 nm) in the presence of sodium acetate[7] and a pronounced downfield shift of the H-8 signal after conversion to a diacetate. Hence the new flavone was 5,7-dihydroxy-3,3′,4′,6-tetramethoxyflavone (III a)



(III)

(a) $R_1, R_2 = H, R_3 = CH_3$
(b) $R_1, R_2, R_3 = CH_3$
(c) $R_1, R_2 = Ac, R_3 = CH_3$
(d) R_1 = glucosyl, $R_2, R_3 = H$
(e) $R_1, R_2, R_3 = H$

Starting material for establishing structure (III a) by synthesis was synthetic jacein, a 4′,5′,7-trihydroxy-3,3′,6-trimethoxyflavone-7-mono-β-D-glucoside, (III d),[9] whose isolation from *Centaurea jacea* L., structure determination and resynthesis from jaceidin (III e) and α-acetobromoglucose was originally reported from one of our laboratories. Subsequently, Fukui *et al.* synthesized jaceidin [10] Jacein was methylated with dimethylsulfate-potassium

* A 2-furoyl group displays its maximum at 245 nm.
† In the NMR spectrum of 2-furoyl derivatives H-5′ appears as a doublet of doublets

[3] A I Scott, *Interpretation of the UV Spectra of Natural Products*, p 137. Pergamon Press, New York
[4] S Gronowitz, G Sorin, B Gestblom and R A Hoffman, *Arkiv Kem* **19**, 483 (1962)
[5] W Stocklin, T. G Waddell and T. A Geissman, *Tetrahedron* **26**, 2397 (1970)
[6] J Massicot and J P Marthe, *Bull Soc Chim Fr* 1962 (1962)
[7] T Batterham and R J Highet, *Austral J Chem* **17**, 428 (1964)
[8] L Jurd, in *The Chemistry of Flavonoid Compounds* (edited by T A Geissman), p 107, McMillan, New York (1962)
[9] L Farkas, L Horhammer, H. Wagner, H. Rosler and R Gurniak, *Chem. Ber* **97**, 610 (1964).
[10] K Fukui, T Matsumoto, S. Nakamura, M Nakayama and T Horie, *Experientia* **24**, 108 (1968)

carbonate in acetone solution Subsequent hydrolysis with methanolic hydrochloric acid furnished the desired 5,7-dihydroxy-3,3',4',6-tetramethoxyflavone, m p 152–153°, which did not depress the m p. of the flavone from *Bahia oppositifolia* R_f-values of both samples were identical and traces of UV, IR and NMR spectra were superimposable Also, the diacetate (II c) of the synthetic flavone had the same m p (176–178°) as the derivative prepared from naturally-occurring material

EXPERIMENTAL*

Extraction of Bahia oppositifolia Dried and ground plant material (wt 0 72 kg) of *Bahia oppositifolia* Nutt , collected by Dr B H Braun in the Denver, Colorado, area in the summer of 1960 when in flower, was extracted with $CHCl_3$ and worked up in the usual manner [11] The crude gum, wt 7 9 g, was chromatographed over 160 g of silicic acid (Mallinckrodt, 100 mesh), 100 ml fractions being collected in the following order Fr 1–6 benzene, 7–11 benzene–$CHCl_3$ (3 1), 12–16 benzene–$CHCl_3$ (1 1), 17–21 benzene–$CHCl_3$ (1 3), 24–28 $CHCl_3$, 29–34 $CHCl_3$–MeOH (97 3), 35–38 $CHCl_3$–MeOH (19 1) and 39–44 $CHCl_3$–MeOH (9 1)

Fr 12 and 13 eluted semicrystalline material Repeated crystallization from EtOAc–hexane gave bahifolin, wt 0 25 g, m p 140–142° Fr 17 and 18 gave 5,7-dihydroxy-3,3',4',6-tetramethoxyflavone (II a) which was repeatedly recrystallized from EtOAc and MeOH, yield 24 mg, m p 152–153° A subsequent recrystallization raised the m p to 158–160° All other fractions were gums showing several spots

Bahifolin (I c) had m p 140–142°, $[\alpha]_D$ + 14 3°, IR bands at 1764, 1720, 1662, 1572 and 858 cm^{-1}, λ_{max} 239 and 204 nm (ϵ 4380 and 27,400), CD curve λ_{max} 255 nm (θ −2200) ($C_{20}H_{20}O_6$ requires C, 67 61, H, 5 66, O, 26 84, mol wt 356 1260 Found C, 67 41, H 5 66, O, 26 94%, mol wt 356 1262)

Flavone (III a) exhibited the following color reactions $FeCl_3$ dark-green to black, Mg–HCl yellow, NaOH yellow, gossypetone test negative It had IR bands at 3488, 1655, 1615, 1593, 1560 and 1510 cm^{-1} and UV maxima (log ϵ) at 255 (4 17), 273 (4 197) and 349 nm (4 235), in EtOH–NaOAc at 361, 315sh and 273 nm, in EtOH–NaOAc–H_3BO_3 at 361, 320sh and 273 nm, in EtOH–NaOMe at 373, 295sh and 276 nm, in EtOH–$AlCl_3$ at 368, 283, 262 and 235sh nm NMR signals ($CDCl_3$) occurred at 12 63 (—OH), 7 69dd (9,2,H-6'), 7 60d (2, H-2'), 6 93d (9, H-5'), 6 50 (H-8), 4 00, 3 92, 3 92, 3 82 ppm (four methoxyls)

A solution of 10 mg (III a) in 1 ml of pyridine and 0 2 ml of Ac_2O was left overnight and worked up in the usual fashion Recrystallization from MeOH afforded 8 mg of diacetate which had m p 177–179°, IR bands at 1772, 1635, 1620, 1558 and 1510 cm^{-1}, NMR signals ($CDCl_3$) at 7 65 and 7 61 (overlapping H-6' and H-2'), 7 23 (H-8), 6 95d (9, H-5'), 3 95, 3 95, 3 86, 3 80 (four methoxyls, 2 50 and 2 30 ppm (two acetates)

A solution of 6 mg of (III a) in 2 ml of dry acetone was refluxed with 0 1 ml of Me_2SO_4 and 0 3 g of K_2CO_3 for 3 days Recrystallization of the product from MeOH afforded 4 mg of (III b), m p 156–158°, m m p up with hexamethylquercetagetin undepressed, IR spectra superimposable

Hydrolysis of Bahifolin A solution of 50 mg of (I c) in 8 ml of 80% aq MeOH was stirred with 40 mg of Na_2CO_3 under N_2 for 2 hr The solvent was removed *in vacuo* and the residue diluted with H_2O and extracted with $CHCl_3$ The washed and dried $CHCl_3$ layer was evaporated and the residue purified by TLC The gummy residue, wt 35 mg, was acetylated overnight with 1 ml of pyridine and 0 5 ml of AC_2O The product was worked up in the usual way, recrystallization from MeOH–H_2O afforded 22 mg of (II) which had m p 95–97°, IR bands at 1770 and 1738 cm^{-1}, NMR signals at 5 50 m (H-3), 5 19 td (8,3, H-8), 4 50 m (H-6), 3 62 (AB quartet, H-13), 3 44 (methoxyl), 2 75 m (H-7), 2 66 (H-14), 1 85 br (vinyl methyl) (calc for $C_{18}H_{24}O_6$ C, 64 27, H, 7 19, O, 28 54 Found C, 64 23, H, 7 16, O, 28 84%)

An authentic sample of (II) was prepared by hydrolysis of (I b) in the manner described in the previous paragraph Authentic (II) had m p 95–97°, m m p with the preceding sample undepressed, NMR spectra superimposable

Synthesis of 5,7-*dihydroxy*-3,3',4',6-*tetramethoxyflavone* (III a) A solution of 300 mg of synthetic jacein (III d)[9] in 30 ml of acetone was refluxed with 75 mg of Me_2SO_4 and 1 g of K_2CO_3 for 10 hr with stirring The solution was filtered and the salts washed with acetone The combined filtrates were evaporated *in vacuo* The residue was taken up in 15 ml of MeOH and 15 ml of 20% HCl and refluxed for 3 hr, cooled and diluted with H_2O The precipitated flavone was recrystallized from EtOH–$CHCl_3$ The yellow needles, yield 110 mg (55%), had m p 152–153°, undepressed on admixture of a sample of naturally-occurring material which had m p 152–153°, λ_{max} 348 (4 315), 272 (4 210) and 254 nm (4 233), TLC R_f 0 68 (silica gel,

* M ps are uncorrected NMR spectra were measured on Varian A-60 or Bruker 90 mHz spectrometers, mass spectra on a Nuclide 12 in medium or a MS-9 high resolution mass spectrometer, CD spectra on a Jasco ORD/UV-5 recording spectrophotometer Elementary analyses were performed by Dr F Pascher, Bonn, Germany

[11] W HERZ and G HOGENAUER, *J Org Chem* 27, 905 (1962)

benzene–pyridine–HCOOH 72 18 10) ($C_{19}H_{18}O_6$ requires C, 60 96; H, 4 84, OCH_3 33 16 Found C, 60 85, H, 4 80, OCH_3 33 07%)

The diacetate was prepared by the AC_2O–NaOAc method and was recrystallized from EtOH–$CHCl_3$, m p 177–178°, λ_{max} 341 (4 355) and 249 nm (4 396), TLC R_f 0 74 (silica gel, benzene–pyridine–HCOOH 72 18 10) ($C_{23}H_{22}O_{10}$ requires C, 60 26, H, 4 83, OCH_3 27 07 Found C, 60 70, H, 4 80, OCH_3 26 43%)

Extraction of Bahia absinthifolia *var* dealbata (*Gray*) *Gray* Above ground parts of *Bahia absinthifolia* Benth var *dealbata* (Gray) Gray, wt 1 4 kg, collected by R J Barr, on May 7, 1963 at the South exit of the Hughes Plant Road, Pima County, Arizona (Barr No 63181 on deposit in herbarium of Florida State University) was extracted with $CHCl_3$ in the usual manner [11] The crude gum, wt 44 5 g, was dissolved in benzene and chromatographed over 350 g of silicic acid, 400 ml fractions being collected and monitored by TLC in the following order Fr 1–25 benzene, fr 26–54 benzene–$CHCl_3$ (1 1), fr 55–95 $CHCl_3$, fr 96–110 $CHCl_3$–MeOH (19 1), fr 111–118 $CHCl_3$–MeOH (9 1)

Fractions 38–41 yielded crystalline material and were combined Repeated recrystallization from EtOAc isopropyl ether afforded 124 mg of bahia I which had m p 206–208°, i r bands ($CHCl_3$) at 3580, 3460, 1765, 1660 and 1600 cm^{-1} Direct comparison with an authentic sample of bahia I[1]* established identity

Fractions 79–106, although gummy, were homogeneous by TLC criteria and showed a tendency to crystallize The material from fr 79–88 was combined, repeated recrystallization from acetone–isopropyl ether afforded 152 mg of bahia II, m p 124–128° (lit [1] 134–137°), IR bands at 3580 and 3410 (OH), 1760, 1720 and 1660 cm^{-1} A mixed m p comparison with a sample of authentic bahia II (m p 133–134°) showed a slight depression, but the IR and NMR spectra were superimposable and TLC behavior was identical Additional quantities of impure bahia II were recovered from fractions 89–106

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* We wish to thank Dr A Romo de Vivar for supplying samples of bahia I and II

Key Word Index—Bahia oppositifolia, Compositae, bahifolin, sesquiterpenoid lactone, 5,7-dihydroxy-3,3′,4′,6-tetramethoxyflavone, flavone