

PRENYLATED COUMARINS FROM THE LEAVES OF *CLAUSENA ANISATA*

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ABSTRACT.—Four new coumarins, anisocoumarins E [1], F [2], G [3], and H [4], have been isolated from the leaves of *Clausena anisata*. Their structures were determined by spectroscopic methods. Three known members of this group of compounds, imperatorin, capnolactone, and triphasiol, were also obtained. All the coumarins carry at least one prenyl group.

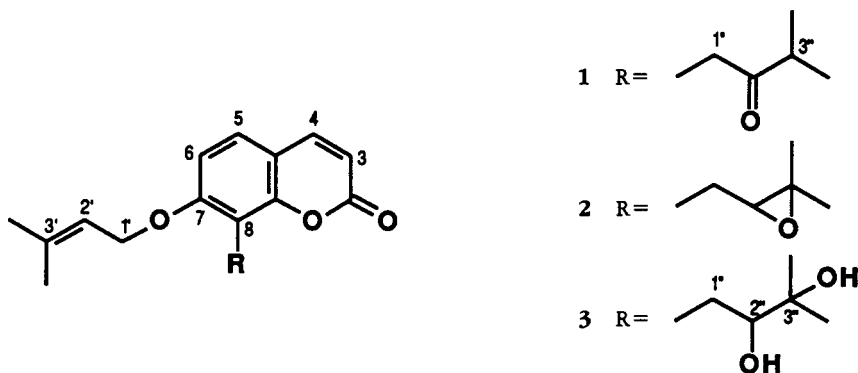
Clausena anisata (Will.) Hook. f. ex Benth. (Rutaceae), whose leaves are widely used in West Africa as an insect repellent (1), is a rich source of coumarins (2,3). Two of the coumarins have been shown to exhibit insect antifeedant activity (4). In a continuing study of this plant (5), we have identified seven coumarins from the leaves, four of which are new. All of these carry at least one prenyl group and differ from each other by the degree of oxidation of the prenyl groups. We present here the isolation and characterization of these compounds.

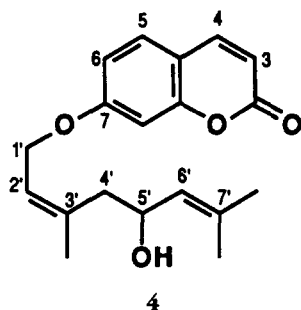
RESULTS AND DISCUSSION

The crude petroleum ether extract of the powdered leaves gave a precipitate on standing. The concentrated filtrate upon successive cc and thick layer preparative chromatography afforded seven compounds in low overall yield (see Experimental). Three of these were identified from published physical and spectroscopic data as the known imperatorin (6), capnolactone (7), and triphasiol (8,9). The four new compounds were named anisocoumarins E [1], F [2], G [3], and H [4]. Anisocoumarins A–D have been reported (5) in our previous study of this plant.

Compounds 1–4 were found to be coumarins by their color reaction with alkaline hydroxylamine followed by FeCl_3 (10) and their uv and ir absorptions. Furthermore, their uv spectra were typical of coumarins oxygenated at C-7 (11). The downfield ^1H -nmr spectral patterns of compounds 1–3 (Table 1) were identical and suggested that they were 7,8-disubstituted coumarin derivatives. The spectra contained four well-resolved doublets, $J = 8.5\text{--}10.0$ Hz at δ 6.16–6.24, 6.80–6.88, 7.30–7.37, and 7.57–7.63, due, respectively, to H-3, H-6, H-5, and H-4.

The other compound 4 was found to be a 7-substituted coumarin by the resonance





signals at δ 6.22 (1H, d, $J = 9.5$ Hz, H-3), 7.62 (1H, d, $J = 9.5$ Hz, H-4), and 7.34 (1H, dd, $J = 8.3$ and 0.5 Hz, H-5). The further splitting of the H-5 signal and the appearance of another doublet of doublets ($J = 2.4$ and 0.5 Hz) at δ 6.79 indicated that C-8 was free.

TABLE 1. ^1H -nmr Spectral Data of Anisocoumarins E [1], F [2], and G [3].^a

Proton	Compound		
	1 ^b	2 ^b	3 ^c
3	6.17 d (10.0)	6.22 d (10.0)	6.21 d (9.5)
4	7.60 d (10.0)	7.61 d (10.0)	7.62 d (9.5)
5	7.32 d (9.0)	7.30 d (9.0)	7.34 d (8.5)
6	6.80 d (9.0)	6.85 d (9.0)	6.88 d (8.5)
1'	4.55 br d (8.0)	4.62 br d (8.0)	4.62 br d (8.0)
2'	5.40 br t (8.0)	5.45 br t (8.0)	5.42 br t (8.0)
1''	—	3.39 dd (14.0, 7.0)	3.00 dd (13.0, 2.0)
1'''	—	3.20 dd (14.0, 6.0)	2.85 dd (13.0, 10.0)
2''	—	3.09 dd (7.0, 6.0)	3.60 br dd (10.0, 2.0)
-CH ₂ CO-	4.00 s	—	—
CH(CH ₃) ₂	2.80 septet (6.0)	—	—
2''-OH	—	—	3.80 br s
3''-OH	—	—	2.25 br s
Me	1.75 br s	1.78 br s	1.79 br s
	1.70 br s	1.73 br s	1.75 br s
	1.20 d (6.0)	1.45 s	1.29 s
	1.12 d (6.0)	1.25 s	1.29 s

^aValues in parentheses are coupling constants (Hz).

^bRecorded at 90 MHz.

^cMeasured at 200.13 MHz.

Anisocoumarins E [1], F [2], and G [3] showed two vinylic methyl singlets at δ_{H} 1.70–1.79 and a broad olefinic triplet ($J = 8.0$ Hz) at δ_{H} 5.40–5.45 of a one-proton coupled to a two-proton doublet ($J = 8.0$ Hz) at δ_{H} 4.55–4.62, which was consistent with a 3-methylbut-2-enyloxy (prenyloxy) group. Compounds 1–3 could therefore be defined as 7-prenyl ethers of coumarins differing from each other only by the nature of the C-8 substituent groups. The C-8 substituents had therefore to be determined. The ^1H -nmr spectra of anisocoumarins E [1], F [2], and G [3] were very informative in this respect; they readily defined the C-8 substituent in 1 as the 3-methyl-2-oxobutyl group [δ_{H} 4.00 (2H, s), 2.80 (1H, septet, $J = 6.0$ Hz), 1.20 (3H, d, $J = 6.0$ Hz), and 1.12 (3H, d, $J = 6.0$ Hz)], that in 2 as the isomeric 2,3-epoxy-3-methylbutyl moiety [δ_{H} 3.39 (1H, dd, $J = 14.0$, $J' = 7.0$ Hz), 3.20 (1H, dd, $J = 14.0$, $J' = 6.0$ Hz), 3.09 (1H, dd, $J = 7.0$, $J' = 6.0$ Hz), 1.45 (3H, s), and 1.25 (3H, s)], and finally that in 3 as

the 2,3-dihydroxy-3-methylbutyl residue [δ_{H} 3.00 (1H, dd, $J = 13.0, J' = 2.0$ Hz), 2.85 (1H, dd, $J = 13.0, J' = 10.0$ Hz), 3.60 (1H, br dd, $J = 10.0, J' = 2.0$ Hz), 3.80 (1H, br s, D_2O exchangeable), 2.25 (1H, br s, D_2O exchangeable), and 1.29 (6H, s)]. Thus, anisocoumarins E–G were defined, respectively, as 7-(3-methylbut-2-enyloxy)-8-(3-methyl-2-oxobutyl) coumarin [**1**], 7-(3-methylbut-2-enyloxy)-8-(2,3-epoxy-3-methylbutyl) coumarin [**2**], and 7-(3-methylbut-2-enyloxy)-8-(2,3-dihydroxy-3-methylbutyl) coumarin [**3**]. Structures **1**, **2**, and **3** were also consistent with their mass spectral fragmentation patterns.

Anisocoumarin H [**4**] was obtained as a colorless oil. Its mass spectrum underwent facile loss of $\text{C}_{10}\text{H}_{16}\text{O}$ to give the base peak m/z 162. The ^1H -nmr spectrum (Table 2) of **4** showed a coumarin system with an additional C_{10} moiety which consisted of three vinyl methyl groups (δ_{H} 1.80, 1.69, 1.67; δ_{C} 17.1, 18.2, and 25.7), two methylenes (δ_{C} 65.3, 47.7), two trisubstituted double bonds (δ_{H} 5.55, 5.15; δ_{C} 138.9, 135.5), three methines (δ_{C} 66.5, 121.8, 128.8), and a one-proton D_2O exchangeable broad singlet δ_{H} 3.52. This C_{10} moiety should be linked at C-7 through an oxygen atom (cf. uv). The above spectroscopic data are best accommodated by structure **4**, 7-(5-hydroxy-3,7-dimethylocta-2,6-dienoxy) coumarin, for anisocoumarin H. The proposed structure **4** was consistent with the ^{13}C -nmr spectrum (Table 2) in which all the carbon atoms were assigned using the DEPT technique. The structure **4** was confirmed by chemical correlation. Jones oxidation of **4** yielded a ketone, $\text{C}_{19}\text{H}_{20}\text{O}_4$, whose mp and spectral data (ir, uv, ^1H nmr) were identical with those reported for 5'-oxoaurapten (12).

TABLE 2. ^1H - and ^{13}C -nmr Chemical Shifts of Anisocoumarin H [**4**].^a

Position	^{13}C nmr ^b	^1H nmr ^c
C-2	162.1 s	—
C-3/H-3	113.1 d	6.22 d (9.5)
C-4/H-4	143.5 d	7.62 d (9.5)
C-4a	112.6 s	—
C-5/H-5	127.4 d	7.34 dd (8.3, 0.5)
C-6/H-6	113.2 d	6.81 dd (8.3, 2.4)
C-7	161.3 s	—
C-8/H-8	101.7 d	6.79 dd (2.4, 0.5)
C-8a	155.9 s	—
C-1'/H-1'	65.3 t	4.59 br d (6.5)
C-2'/H-2'	128.8 d	5.55 tq (6.5, 1.2)
C-3'	138.9 s	—
C-4'/H-4'	47.7 t	2.25 dd (13.8, 7.3) 2.20 dd (13.8, 6.1)
C-5'/H-5'	66.5 d	4.51 ddd (7.3, 6.1, 5.4)
C-6'/H-6'	121.8 d	5.15 dq (7.3, 1.4)
C-7'	135.5 s	—
3'-Me	17.1 q	1.80 br d (1.2)
7'-Me	18.2 q	1.69 d (1.4)
	25.7 q	1.67 d (1.4)
OH	—	3.52 br s

^aValues in parentheses are coupling constants (Hz).

^b25.2 MHz.

^c200.13 MHz.

The isolation of anisocoumarins E–H adds to the already long list of coumarins isolated from *C. anisata* (2,3). A noteworthy characteristic of all these coumarins is the presence of either C- or O-prenylated functions of various degrees of oxidation in their homocyclic rings.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were taken on a Kofler hot stage equipped with a microscope. Uv spectra were recorded with a Beckman model 25 grating spectrophotometer and ir spectra on a Perkin-Elmer model 727B instrument. Optical rotations were measured on an AA-100 automatic polarimeter. ^1H -nmr measurements were made in CDCl_3 solutions, unless otherwise mentioned, on a Perkin-Elmer R12 or a Bruker WP200SY instrument, with TMS as an internal standard, and all the signals are reported as δ values. ^{13}C -nmr spectra were determined in CDCl_3 solutions (which also provided the lock signal) on a Varian XL-100 spectrometer with VFT-100 accessory, and the chemical shifts are reported in ppm downfield from TMS. Eims were obtained at 70 eV by direct inlet. Flash cc was conducted on Merck Si gel 60H-254 (70–230 mesh). Tlc and ptlc of compounds were accomplished on Merck Si gel 60 F₂₅₄, and the spots were visualized in uv light (254 or 366 nm).

PLANT MATERIAL.—The leaves of *C. anisata* were collected at Oku, 180 km from Bamenda, North West Province of Cameroon, in April 1987 by Benoit Mpom. The plant material was confirmed by the Cameroon National Herbarium, Yaounde, where a voucher specimen has been deposited.

EXTRACTION AND CHROMATOGRAPHIC SEPARATION.—The powdered sun-dried leaves (1 kg) were extracted exhaustively with petroleum ether (bp 60–80°) (5 liters), and the solution was concentrated to a volume of 500 ml. The solution upon cooling and centrifugation gave a precipitate which was collected by filtration. The filtrate was evaporated to dryness in vacuo and the dark green residue (40 g) dissolved in CHCl_3 (30 ml) and fractionated on a Si gel column eluted with petroleum ether/EtOAc mixtures and finally with 5–10% MeOH in EtOAc. The eluate was collected in 250-ml fractions, and the fractions were combined on the basis of tlc comparison with an appropriate solvent system and in some cases with the aid of ^1H nmr. A total of 115 fractions were collected. The precipitate (10 g) proved (^1H nmr, ir) to be a hydrocarbon.

ISOLATION AND CHARACTERIZATION.—From the chromatographic separation above, and with the aid of flash cc and preparative tlc a total of seven coumarins were obtained. They are presented in order of elution from the column.

Anisocoumarin E [1].—Obtained as a pale yellow oil (75 mg) from the preparative tlc of combined fractions 20–28: uv λ max EtOH (log ϵ) 246 sh (3.53), 260 sh (3.69), 325 (4.12) nm; ir ν max (CHCl_3) 1715, 1700, 1605, 1560, 1485, 1455, 1430, 1400, 1380, 1280, 1260, 1240, 1115, 1090, 1065, 900, 825 cm^{-1} ; ^1H nmr see Table 1; ^{13}C nmr (CDCl_3 , 25.2 MHz) δ 160.0 (C-2), 113.4 (C-3), 143.9 (C-4), 112.7 (C-4a), 127.2 (C-5), 114.9 (C-6), 159.5 (C-7), 108.4 (C-8), 153.3 (C-8a), 65.7 (C-1'), 121.4 (C-2'), 138.6 (C-3'), 18.4 (C-4'), 25.7 (C-5'), 34.8 (C-1''), 210.7 (C-2''), 40.8 (C-3''), 18.4 (2) (C-Me); eims m/z (rel. int.) $[\text{M}]^+$ 314 (7), 246 (52), 177 (13), 176 (97), 175 (32), 146 (13), 91 (13), $[\text{Me}_2\text{CHCO}]^+$ 71 (40), $[\text{Me}_2\text{C}=\text{CHCH}_2]^+$ 69 (75), 43 (89). Found C 72.50, H 7.0; $\text{C}_{19}\text{H}_{22}\text{O}_4$ requires C 72.59, H 7.06%.

Anisocoumarin F [2].—Recovered from combined fractions 30–42. Further purification by preparative tlc afforded **2** as a yellow oil (30 mg): $[\alpha]^{25}_{\text{D}} + 27.5^\circ$ ($c = 1.5$, CHCl_3); uv λ max EtOH (log ϵ) 248 sh (3.66), 259 (3.69), 324 (4.05) nm; ir ν max (CHCl_3) 1720, 1605, 1485, 1430, 1400, 1375, 1280, 1240, 1110, 920, 830 cm^{-1} ; ^1H nmr see Table 1; eims m/z (rel. int.) $[\text{M}]^+$ 314 (10), $[\text{M} - \text{C}_5\text{H}_8]^+$ 246 (20), 213 (20), 201 (11), 189 (11), 188 (23), 187 (39), 176 (64), 175 (63), $[(\text{CH}_3)_2\text{C}=\text{CH}]^+$ 71 (23), 69 (51). Found C 72.55, H 7.10; $\text{C}_{19}\text{H}_{22}\text{O}_4$ requires C 72.59, H 7.06%.

Anisocoumarin H [4].—Flash chromatography and preparative tlc with CHCl_3 -MeOH (97:3) on combined fractions 61–73 yielded a colorless oil (25 mg): $[\alpha]^{25}_{\text{D}} - 20.5^\circ$ ($c = 1.04$, CHCl_3); uv λ max EtOH (log ϵ) 255 sh (3.42), 298 sh (3.86), 324 (4.08); ir ν max (CHCl_3) 3540, 1725, 1625, 1580, 1560, 1470, 1400, 1380, 1270, 1245, 1100, 1050, 1000, 900 cm^{-1} ; ^1H - and ^{13}C -nmr spectral data, see Table 2; eims m/z (rel. int.) $[\text{M}]^+$ 314 (5), $[\text{M} - \text{H}_2\text{O}]^+$ 296 (28), $[\text{M} - \text{C}_4\text{H}_7]^+$ 259 (16), 163 (20), $[\text{M} - \text{C}_{10}\text{H}_{16}\text{O}]^+$ 162 (100), 134 (37), 133 (13), 105 (18), 85 (80). Found C 72.70, H 7.10; $\text{C}_{19}\text{H}_{22}\text{O}_4$ requires C 72.59, H 7.06%.

Oxidation of anisocoumarin H [4].—Jones reagent (8 drops) was added to a solution of **4** (10 mg) in MeOH (3 ml). The mixture was allowed to stand at room temperature for 30 min. The usual work up gave crude 5'-oxoaurapten which was purified by crystallization in Et_2O to give 7-(5-oxo-3,7-dimethylocta-2,6-dienoxy)coumarin (6 mg): mp 61–62° [lit. (12) mp 63.5°]; ir ν max (CHCl_3) 1725, 1680, 1625; ms and ^1H -nmr spectra superimposable with those reported for 5'-oxoaurapten (12).

Anisocoumarin G [3].—Yellow oil, $[\alpha]^{25}_{\text{D}} + 32.5^\circ$ ($c = 1.07$, CHCl_3); uv λ max EtOH (log ϵ) 238 sh (3.67), 250 (3.62), 260 (3.62), 325 (4.09) nm; ir ν max (CHCl_3) 3550, 3400, 1720, 1705, 1605, 1560, 1490, 1460, 1430, 1400, 1380, 1275, 1240, 1160, 900, 830 cm^{-1} ; ^1H nmr see Table 1; eims m/z (rel. int.) $[\text{M}]^+$ 332 (8), 264 (12), 246 (12), 206 (13), 205 (24), 176 (33), 175 (24), $[\text{C}_5\text{H}_{10}\text{O}_2]^+$ 102 (25), 91

(17), 71 (10), 69 (57), [Me₂COH]⁺ 59 (62). Found C 68.50, H 7.20; C₁₉H₂₄O₅, requires C 68.65, H 7.28%.

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