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2*R*,3*R*-(+)-3-Acetoxy-4′,5-dihydroxy-7-methoxyflavanone and 2*R*,3*R*-(+)-3-Acetoxy-4′,5,7-trihydroxyflavanone: Two New 3-Acetylated dihydroflavonols from *Aframomum pruinosum* Gagnepain (Zingiberaceae)

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Two new 3-acetylated dihydroflavonols, 2*R*,3*R*-(+)-3-acetoxy-4′,5-dihydroxy-7-methoxyflavanone (1) and 2*R*,3*R*-(+)-3-acetoxy-4′,5,7-trihydroxyflavanone (2), have been obtained from the seeds of *Afromomum pruinosum* Gagnepain, together with the known 4′,7-di-*O*-methylaromadendrin (5). Nerolidol (8) was also present in substantial amounts. The ^{13}C n.m.r. resonances of these compounds have been assigned.

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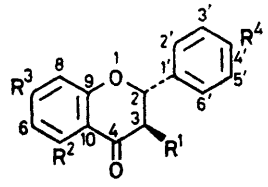
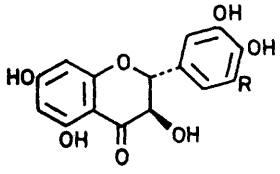
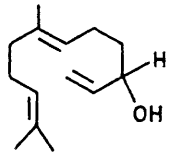
As part of a continuing programme of chemical studies on Cameroonian *Aframomum* species (Zingiberaceae) of medicinal value,[1] extractives of the seeds of *Aframomum pruinosum*[2] have been studied and three flavonoids have been isolated. Two of them, 2*R*,3*R*-(+)-3-acetoxy-4′,5-dihydroxy-7-methoxyflavanone (1) and 2*R*,3*R*-(+)-3-acetoxy-4′,5,7-trihydroxyflavanone (2), are new and the third is the rare naturally occurring 4′,7-di-*O*-methylaromadendrin (5).[3]



(1) R^1 = OAc, R^3 = OMe, R^2 = R^4 = OH
(2) R^1 = OAc, R^2 = R^3 = R^4 = OH
(3) R^1 = R^3 = R^4 = OAc, R^2 = OH
(4) R^1 = OAc, R^2 = R^3 = R^4 = OMe
(5) R^1 = R^2 = OH, R^3 = R^4 = OMe
(6) R^1 = R^2 = R^4 = OH, R^3 = OMe



(7) R = H
(9) R = OH



(8)

Crystallisation of the pale yellow solid which precipitated from the concentrated hexane extract of the seeds afforded the first compound, (+)-3-acetoxy-4′,5-dihydroxy-7-methoxyflavanone (1), $C_{18}H_{16}O_7$ [$\nu_{max.}$ 3 320, 1 640, and 1 720 cm^{-1}] which gave the colour reactions of a flavanone.[4] The ^1H n.m.r. spectrum revealed signals for an acetate (δ 2.01, 3H, s), a methoxy-group (δ 3.81, 3 H, s), two phenolic hydroxy-groups, (δ 11.45 and 5.55, exchangeable with D_2O), a 4′-substituted ring B (AA′BB′, δ 6.85 and 7.35, *J* 9 Hz), a 5,7-disubstituted ring A (δ 6.06 and 6.14, ABq, *J* 2 Hz), H-2 (δ 5.30, d, *J* 12 Hz) and H-3 (δ 5.85, d, *J* 12 Hz). The lowfield chemical shift of H-3 and its large coupling constant indicated that the acetate was attached equatorially to C-3 with a *trans*-relationship to the substituent on C-2. The methoxy-group was placed at C-7 on the basis of the u.v. spectrum. Addition of sodium acetate did not cause the usual bathochromic shift of band II, expected for 7-hydroxyflavonoids.[5] The above data confirmed the structure of the first compound as (+)-3-acetoxy-4′,5-dihydroxy-7-methoxyflavanone (1).

The second compound, (+)-3-acetoxy-4′,5,7-trihydroxyflavanone (2), $C_{17}H_{14}O_7$ [$\nu_{max.}$ 3 300—3 100, 1 640, and 1 725 cm^{-1}], isolated from the chloroform extract, was the major component of the seeds. Its u.v. spectroscopic data (see Experimental section) together with colour tests with ferric chloride (brown-red) and magnesium–concentrated hydrochloric acid (pink)[4] indicated that it was a flavanoid. The ^1H n.m.r. spectrum revealed that it was a 2,3-*trans*-3-acetoxyflavanoid [δ 1.98 (3 H, s, OAc), 5.77 (1 H, d, *J* 12 Hz, H-3), and 5.25 (1 H, d, *J* 12 Hz, H-2)] and confirmed the presence of the same oxygenation pattern as in (1) [δ 6.85 and 7.27 (AA′BB′, *J* 9 Hz) and 5.98 and 6.04 (ABq, *J* 2 Hz, H-6 and H-8)]. Thus the second compound was (+)-3-acetoxy-4′,5,7-trihydroxyflavanone (2) (3-acetylaromadendrin). Acetylation of (2) with acetic anhydride in pyridine afforded 3,4′,7-triacetylaromadendrin (3) while methylation with methyl iodide and anhydrous potassium iodide in dry acetone yielded the tri-*O*-methyl derivative (4) which gave a negative ferric chloride test. The mass spectral fragmentation pattern of compound (2)[6] and its ^{13}C n.m.r. spectrum were also in accord with the proposed structure.

The third flavanoid, obtained by chromatography of the hexane extract after the isolation of (1), was identified as 4,7-di-*O*-methylaromadendrin (5).[3,7] Although its m.p. was slightly higher than the one recorded in literature,[3,7] the ^1H n.m.r. spectrum was as expected and the u.v. spectrum duplicated the one recorded[3] for (5). No authentic sample of (5) was available for direct comparison. The ^{13}C n.m.r. resonances of the three aromadendrin derivatives have been assigned and are recorded in the Table.

All naturally occurring 3-hydroxyflavanones have the

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^{13}C N.m.r. data [a] for aromadendrin derivatives, taxifolin (7), and ampelopsin (9)

	(1) [b]	(2)	(5)	(7)	(9)
2	81.7	80.2	82.7	83.1	83.2
3	72.9	72.0	71.6	71.6	71.6
4	192.6	191.5	198.3	197.5	197.5
5	164.2	163.4	163.0	163.3	162.4
6	95.9	96.6	94.9	96.1	94.9
7	169.1	167.4	167.6	166.8	166.7
8	95.0	95.5	93.8	95.0	95.9
9	162.9	162.4	162.4	162.5	163.3
10	102.2	100.7	101.3	100.5	100.4
1′	126.5	125.8	129.2	128.1	127.1
2′	129.3	129.2	129.4	115.4	106.9
3′	115.9	115.3	113.6	144.9	145.6
4′	158.5	158.2	159.6	145.7	133.4
5′	115.9	115.3	113.6	115.4	145.6
6′	129.3	129.2	129.4	119.5	106.9
Ac	170.3	168.7			
	20.3	20.0			

[a] Pulse Fourier transform spectra were obtained at 25.2 MHz from solutions in $(CD_3)_2SO$ at room temperature. Peaks are given as positive downfield from internal Me_4Si. Assignments are based on chemical-shift rules and correlations with ^{1}H chemical shifts using two off-resonance decoupled spectra and comparison with ampelopsin (9) and published data for taxifolin (7) (P. J. Cotterill, F. Scheinmann, and I. A. Stenhouse, *J. Chem. Soc., Perkin Trans. 1*, 1979, 52). [b] $CDCl_3$ + CD_3OD.

trans-2*R*,3*R*-configuration.[8] The diequatorial relationship of the 2-aryl and 3-acetyl substituents of (1), (2), and (5) was evident from their ^{1}H n.m.r. spectra, all of which exhibited $J_{2,3}$ = 12 Hz. The absolute configuration (2*R*,3*R*) of compounds (1), (2), and (5) was confirmed by comparison of their c.d. spectra (see Experimental section) with that of 7-*O*-methylaromadendrin (6) whose absolute stereochemistry has been shown to be 2*R*,3*R*.[9]

Vaccuum distillation of the non-polar oils from chromatography of the hexane extract afforded a sweet smelling liquid, identified as nerolidol (8), previously reported [10] by Sutherland in 1960. Its structure was determined by a combination of ^{1}H and ^{13}C n.m.r. spectroscopy (see Experimental section), and mass spectrometry.

Acetylated 3-hydroxyflavanones are very rare. To our knowledge, the only known member of this group is 3-acetylalpinone,[11] isolated from *Alpinia*, another Zingiberaceae. The occurrence of these compounds uniquely in the Zingiberaceae may have some biosystematic significance.

EXPERIMENTAL

M.p.s were measured with a Kofler hot-stage apparatus and are uncorrected. N.m.r. (^{13}C and ^{1}H) spectra were obtained on a Varian XL-100 instrument with VFT-100 accessory or a Perkin-Elmer R12B, using solutions in [$^2H_6$]-dimethyl sulphoxide with tetramethylsilane as internal standard. I.r. spectra were run as KBr discs on a Perkin-Elmer 727 B and u.v. spectra on a Beckmann 25. Mass spectra were obtained on an AEI MS12 single focusing instrument. Specific rotations refer to chloroform solutions and were run on an automatic Perkin-Elmer 141 polarimeter. Elemental analyses were determined in the Institute of Chemistry, Strasbourg, by Dr. M. Hemmert.

Extraction.—Dried seeds (1 kg) of *A. pruinosum*, collected from Abong-Mbang in the Eastern Province of Cameroon in November 1979 were powdered, and extracted in a Soxhlet successively with hexane and chloroform for 24 h. The hexane extract was concentrated to leave a brown oil which was set aside for 2 days. The deposited solid was filtered off and washed repeatedly with hexane to give (1) (300 mg). Recrystallisation from methanol–hexane gave the pure 2R,3R-(+)-3-*acetoxy*-4′,5-*dihydroxy*-7-*methoxyflavanone* (1), m.p. 203—204 °C, $[\alpha]_D$ +13° (EtOH, *c* 0.2); c.d. (MeOH) $\Delta\varepsilon_{328}$ + 4.10, $\Delta\varepsilon_{291}$ − 15.0, $\Delta\varepsilon_{254}$ + 2.9, and $\Delta\varepsilon_{235}$ + 4.08; $\lambda_{max.}$ (ε) 217 (25 000), 229 (23 000), 292 (17 000), and 333sh nm (4 000); $\lambda_{max.}$ (EtOH–NaOAc) 217 (25 000), 228 (22 000), 290 (16 200), and 332 (4 000); *m/e* 344 (M^+), 302, 284, 244, 201, 167, and 194 (Found: C, 62.9; H, 4.5. $C_{18}H_{16}O_7$ requires C, 62.79; H, 4.65%).

Isolation of 2R,3R-(+)-3-*Acetoxy*-4′,5,7-*trihydroxyflavanone*.—The chloroform extract on concentration afforded a semisolid (23 g), which was chromatographed over silica gel (650 g). Gradient elution was effected with benzene–ethyl acetate mixtures. 73 Fractions were collected and mixed on the basis of t.l.c. and ^{1}H n.m.r. spectroscopic data. Fractions 18—23 yielded more of compound (1) (320 mg). Fractions 52—60 were rechromatographed on a short column of silica gel. Benzene–ethyl acetate (4 : 6) eluted 2R,3R-(+)-3-*acetoxy*-4′,5,7-*trihydroxyflavanone* (2) (11 g), m.p. 241—242 °C (from methanol–chloroform), $[\alpha]_D$ +24° (EtOH, *c* 0.6), $\lambda_{max.}$ (ε) (EtOH) 218 (22 500), 295 (14 000), and 333 nm (9 100); $\lambda_{max.}$ (ε) (EtOH–NaOAc) 334 (21 000); *m/e* 330 (M^+), 303, 288, 270, 167, 153, 149, 136, 134, and 107 (Found: C, 62.0; H, 4.05. $C_{17}H_{14}O_7$ requires C, 61.82; H, 4.24%).

3,4′,7-*Triacetoxyaromadendrin* (3).—A solution of compound (2) (300 mg) in pyridine (5 ml) was treated with acetic anhydride (2.5 ml) and left at room temperature for 72 h. The reaction mixture was poured onto crushed ice and extracted with methylene chloride; the extract was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated to leave a gum. Filtration of this through a short column of silica gel afforded 3,4′,7-*triacetoxyaromadendrin* (3) (160 g) which recrystallised from ethyl acetate–hexane as needles, m.p. 180—181 °C, $\nu_{max.}$ 3 300—3 100, 1 760, 1 740, 1 675, 1 620, 1 580, 1 507 and 1 210 cm^{-1}; c.d. (MeOH) $\Delta\varepsilon_{339}$ + 0.63 and $\Delta\varepsilon_{275}$ − 1.31; $\lambda_{max.}$ (ε) (EtOH), 228 (30 700), 284 (19 000), and 333 nm (5 500); $\lambda_{max.}$ (EtOH–NaOAc) 228sh, 282, and 336 nm; δ 1.99 (3 H, s, 3-OAc), 2.23 (3 H, s 4′-OAc), 2.26 (3 H, s, 7-OAc), 5.40 (1 H, d, *J* 12 Hz, H-2), 5.79 (1 H, d, *J* 12 Hz, H-3), 6.28, and 6.37 (each 1 H, d, *J* 2 Hz, H-6 and H-8), and 7.14 and 7.45 (each 2 H, d, *J* 9 Hz, H-2′, H-3′, H-5′, and H-6′) (Found: C, 70.85; H, 4.4. $C_{21}H_{18}O_9$ requires C, 60.87; H, 4.38%).

3-*Acetoxy*-4′,5,7-*tri*-O-*methylaromadendrin* (4).—3-Acetoxyaromadendrin (2) (200 mg) was dissolved in anhydrous acetone (10 ml), and anhydrous potassium carbonate (0.8 g) and methyl iodide (5 ml) were added to it. The mixture was heated on a water-bath with constant stirring for 5 h. The resulting 3-*acetoxy*-4′,5,7-*tri*-O-*methylaromadendrin* was purified by preparative t.l.c. Pure crystalline compound (4) (80 mg) was obtained by recrystallisation from ethyl acetate–hexane and had m.p. 152—153 °C, $\nu_{max.}$ 1 738, 1 685, 1 605, and 1 505 cm^{-1}; $\lambda_{max.}$ (ε) (EtOH) 228 (17 000), 282 (11 000), and 322 nm (3 000); (EtOH–NaOAC) 227 (17 000), 282 (11 000), and 322 nm (3 200); δ 1.94 (3 H, s, 3-OAc), 3.78 (9 H, s, OMe's), 5.27 (1 H, d, *J* 12 Hz, H-2), 5.57 (1 H, d, *J* 12 Hz, H-3), 6.07 and 6.18 (each 1 H, d, *J* 2 Hz, H-6 and H-8), and 6.77 and 7.38 (2 H each, d, *J* 9 Hz, H-2′, H-3′,

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H-5′, and H-6′) (Found: C, 65.0; H, 5.2. $C_{20}H_{20}O_7$ requires C, 64.51; H, 5.38%).

Isolation of 4′,7-Di-O-methylaromadendrin (5).—The mother liquor which remained after isolation of 3-acetoxy-4′,5-dihydroxy-7-methoxyflavanone (1) from the hexane extract, was further concentrated to leave an oil (36 g) which was chromatographed over silica gel (550 g). Elution with hexane–ether (9 : 1) gave a solid (102 mg) which was recrystallised from methanol–hexane to yield needles of *4′,7-di-O-methylaromadendrin* (5), m.p. 194—196 °C (lit.,[7] 187—188 °C), $[\alpha]_D$ +86 ° (pyr, *c* 0.2); c.d. (MeOH) $\Delta\varepsilon_{330}$ + 3.16, $\Delta\varepsilon_{291}$ − 10.4, $\Delta\varepsilon_{254}$ + 2.80, and $\Delta\varepsilon_{232}$ + 4.75; $\nu_{max.}$ 3 470, 1 638, 1 560, and 1 508 cm^{-1}; $\lambda_{max.}$ (ε) (EtOH) 224 (27 000), 292 (16 500), and 333sh nm (4 000); $\lambda_{max.}$ (EtOH–NaOAc) 224 (27 000), 292 (16 500), and 333sh nm (4 000); δ 3.82 (6 H, s), 4.50 (1 H, d, *J* 12 Hz, H-3), 5.12 (1 H, d, *J* 12 Hz, H-2), 6.00 and 6.07 (each 1 H, d, *J* 2 Hz, H-6 and H-8), 6.95 (2 H, d, *J* 9 Hz), and 7.45 (2 H, d, *J* 9 Hz); *m/e* 316 (M^+), 298, 259, 179, 167, 150, 148, 135, 121, and 77 (Found: C, 64.5; H, 5.2. $C_{17}H_{16}O_6$ requires C, 64.56; H, 5.06%).

Nerolidol (8).—The sweet smelling oil from the early fractions of the hexane extract chromatogram were combined and distilled under reduced pressure. The fraction collected at 125 °C, 2 mmHg gave nerolidol (8), n_D^{27} 1.4701 (lit.,[11] b.p. 123 °C at 3 mmHg; n_D^{25} 1.4769); *m/e* 222 (M^+) and 204 (M^+ − 18); δ ^{13}C 145.3 (d), 135.0 (s), 131.0 (s), 124.5 (d), 124.5 (d), 111.6 (t), 73.3 (s), 42.3 (t), 39.8 (t), 26.8 (t), 22.8 (t), 27.7 (q), 25.7 (q), 17.6 (q), and 16.0 (q).

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