Rautandiols A and B, Pterocarpans and Cytotoxic Constituents from *Neorautanenia mitis*[⊥,1]

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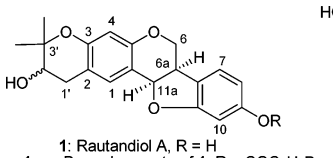
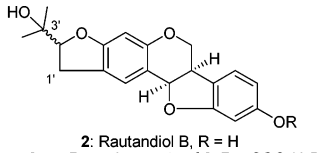
As part of a study on potential antitumor agents from rainforest plants, two new pterocarpans, rautandiol A (**1**) and rautandiol B (**2**), together with eight known compounds, were isolated from *Neorautanenia mitis*. Among the compounds isolated, rotenone (**3**) and 12-hydroxyrotenone (**4**) showed significant cytotoxic activity with IC_{50} values of 0.008−0.010 and 0.04−0.06 μg/mL against MCF-7 and A-549 cells, respectively.

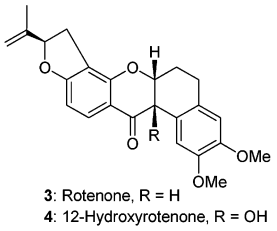
Neorautanenia species of the Fabaceae are widespread in central and south Africa. These plants are used by indigenous populations as fish poisons, as insecticides, and to treat syphilis.[2−4] Several chemical studies on *Neorautanenia* species, including *N. edulis*,[5,6] *N. pseudopachyrrhiza*,[7,8] and *N. amboensis*,[9−12] and on the closely related *Pachyrrhiza erosus*[13−15] have been reported previously. A study on *Neorautanenia mitis* in 1987[16a,b] led to the purification of dehydroneotenone, dolineone, 12-hydroxydolineone, 12-hydroxyrotenone, neodulin, neotenone, and pachyrrhizine. These compounds were also screened for insecticidal and ascaricidal activities. A pharmacological study of an aqueous extract of this plant was also conducted by a Nigerian group.[17]

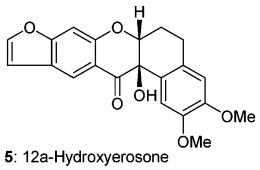
In the course of our efforts to discover antitumor agents from natural sources, *Neorautanenia mitis* (A. Rich) Verdcourt was selected on the basis of its promising preliminary screening results. Herein we describe the isolation of two novel pterocarpans named rautandiols A (**1**) and B (**2**) from the EtOAc-soluble fraction of *N. mitis*. The structures of **1** and **2** were elucidated by detailed 2D NMR analysis and contain dimethylhydroxydihydropyran and hydroxyisopropyldihydrofuran moieties, respectively. Three known rotenoids [rotenone (**3**), 12-hydroxyrotenone (**4**), 12-hydroxyerosone (**5**)], two known isoflavonoids [dehydroneotenone, isoelliptol (**6**)], two known pterocarpans [2-hydroxypterocarpin, neodulin], and one known coumarin [pachyrrizine (**7**)] were isolated also. Compounds **3**,[18−20] **5**,[4,13] **6**,[21] and 2-hydroxypterocarpin[22−24] were isolated for the first time from this plant, while compounds **4**,[25] **7**, dehydroneotenone, and neodulin were previously isolated from *N. mitis*.[16a] All fractionation and purification steps were guided by bioactivity screening. These compounds were evaluated for growth inhibition of the MCF-7 and A-549 human cancer cell lines.

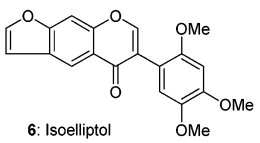
Compound **1** was recrystallized from $CHCl_3$ to give fine colorless needles. Its molecular formula was determined to be $C_{20}H_{20}O_5$, on the basis of HRSIMS data (*m*/*z* 341.1390 $[M + H]^+$). The ^1H NMR spectrum of **1** showed signals at δ 4.18 (1H, dd), 3.51 (1H, dd), 3.50−3.46 (1H, m), and 5.44 (1H, d), which were consistent with the presence of a pterocarpan skeleton. These signals and their splitting patterns were also comparable with those of 2-hydroxypterocarpin and neodulin, which were obtained in the present investigation (Table 1). Two sets of aromatic protons were also present for a 1,2,4-trisubstituted benzene [δ 7.06 (1H, d), 6.31 (1H, dd), and 6.23 (1H, d)] and a 1,2,4,5-tetrasubstituted benzene [δ 7.17 (1H, s) and 6.26 (1H, s)], respectively. The ^{13}C NMR spectrum showed 20 carbons, of which 15 were attributed to the pterocarpan

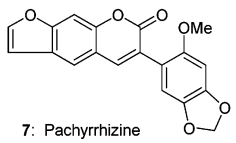












skeleton. The remaining five carbon signals [δ 78.4 (C), 70.6 (CH), 31.6 (CH_2), 26.0 (CH_3), and 21.0 (CH_3)] and the corresponding proton signals [δ 3.74 (1H, dd), 3.00 (1H, dd), 2.69 (1H, dd), 1.33 (3H, s), and 1.24 (3H, s)] were typical of a 3-hydroxy-2,2-dimethyldihydropyran moiety.[26,27] The oxygen atom of the dihydropyran ring was assigned at C-3 due to the upfield shift of the H-4 proton singlet (δ 6.26). Four of the five oxygens were thus determined. As compound **1** gave a *p*-bromobenzoate (**1a**), the remaining oxygen was present as a hydroxyl group and assigned at C-9 of ring D on the basis of the following observations. The H-10 proton signal at δ 6.23 was shifted upfield (δ 6.70 in the ester) and showed a *meta* coupling constant of 2.4 Hz, suggesting that the neighboring C-9 bears a hydroxyl group. The ring arrangement of **1** was confirmed by HMBC correlations from H-1 to C-3, C-4a, C-11a, and C-1′, from H-7 to C-6a, and from H-10 to C-7a. This structure was further supported by the NOESY spectrum of **1**. The absolute stereochemistry at C-6a and C-11a was *R*, as indicated by a negative optical rotation value.[28] However, the configuration at C-2′ was not resolved. From the above data, the structure of compound **1** was represented as formula **1** (6a-*R* and 11a-*R*), named rautandiol A.

Compound **2** was recrystallized from $CHCl_3$ to give fine colorless needles with the same molecular formula, $C_{20}H_{20}O_5$, as **1** based on its HRSIMS (*m*/*z* 341.1389 $[M + H]^+$). Also, like **1**, the ^1H NMR spectrum of **2** showed characteristic signals of a pterocarpan skeleton [δ 5.43 (1H, d), 4.19 (1H, dd), 3.50 (1H, dd), and 3.44−3.48 (1H, m)], as shown in Table 1. Comparison of the aromatic proton region of the ^1H NMR spectrum of **2** [δ (7.24, s), (7.06, d),

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Table 1. ^{1}H NMR Spectroscopic Data of **1** and **2**[a]

position	**1** (CD_3OD)	HMBC	NOESY	**2** (CD_3OD)
1	7.17 s	C-3, 4a, 11a, 1′	H-11a, 1′	7.24 s
4	6.26 s	C-1a, 2, 3, 4a		6.26 s
6eq	4.18 dd (9.6, 3.1)	C-4a, 6a, 11a	H-6a, 7	4.19 dd (10.0, 3.6)
6ax	3.51 dd (10.5, 9.6)	C-6a	H-6a, 7	3.50 dd (10.0, 10.0)
6a	3.50−3.46 m	C-6, 7a	H-6, 7, 11a	3.48−3.44 m
7	7.06 d (8.1)	C-6a, 9, 10a	H-6, 6a, 8	7.06 d (8.1)
8	6.31 dd (8.1, 2.4)	C-7a, 10	H-7	6.31 dd (8.1, 2.2)
10	6.23 d (2.4)	C-8, 9, 10a		6.23 d (2.2)
11a	5.44 d (6.6)	C-1, 1a, 4a, 6	H-1, 6, 6a	5.43 d (6.6)
1′	3.00 dd (16.5, 5.3)	C-1, 2, 3, 2′, 3′	H-1, 1′, 2′, 5′	3.13 d (8.9)
	2.69 dd (16.5, 7.8)	C-1, 2, 3, 2′, 3′	H-1, 1′, 2′, 4′	
2′	3.74 dd (7.8, 5.3)	C-2, 4′	H-1′, 4′, 5′	4.60 t (8.9)
4′	1.33 s	C-2′, 3′, 5′	H-1′, 2′, 5′	1.24 s
5′	1.24 s	C-2′, 3′, 4′	H-1′, 2′, 4′	1.21 s

[a] Values in parentheses are coupling constants (J) in Hz; s = singlet, d = doublet, m = multiplet.

Table 2. Cytotoxicity Data for Compounds Isolated from *N. mitis*[a]

	cell line/IC_{50} (μg/mL)	
compound	MCF-7 breast cancer	A-549 lung cancer
3	0.008	0.04
4	0.010	0.06
5	1.8	3.3
6	0.8	1.6
7	3.6	>5

[a] The new compounds, **1** and **2**, and the known compounds dehydroneotenone, 2-hydroxypterocarpin, and neodulin were not active in this assay system (IC_{50} >5 μg/mL).

(6.31, dd), (6.26, s), and (6.23, d)] with that of **1** revealed that both compounds have the same substitution patterns in rings A and D. The ^{13}C NMR spectra showed that both compounds have identical carbon numbers. The five carbon signals not attributable to the pterocarpan skeleton of **2** were present at δ 72.5 (C), 91.4 (CH), 30.8 (CH_2), 25.4 (CH_3), and 25.2 (CH_3), and the corresponding proton signals were present at δ 4.60 (1H, dd), 3.13 (2H, d), 1.24 (3H, s), and 1.21 (3H, s). These data indicated the presence of a hydroxyisopropyldihydrofuran moiety attached to ring A.[24,29,30] This ring was positioned at C-2 and C-3 of ring A, and again, since an upfield shifted aromatic proton singlet at δ 6.26 (H-4) was observed, the oxygen atom of the dihydrofuran ring was placed at C-3. The hydroxyl group at C-9 of ring D was established similarly to **1**; compound **2** gave a *p*-bromobenzoate (**2a**) and the C-10 proton was shifted upfield (δ 6.23 vs δ 6.70) with a *meta* coupling constant of 2.2 Hz. The same HMBC and NOESY correlations as found in **1** and a negative optical rotation confirmed the ring arrangement and stereochemistry of C-6a and C-11a of **2**. Thus, the structure of compound **2** was represented as formula **2** (6a-*R* and 11a-*R*) and named rautandiol B.

Compounds **1**−**10** were assayed for cytotoxic activity in human MCF-7 breast and A-549 lung cancer cell lines using a reported procedure.[31] The results are shown in Table 2. Among all 10 compounds, rotenone (**3**) and 12-hydroxyrotenone (**4**) were the most potent, with IC_{50} values of 0.008−0.010 and 0.04−0.06 μg/mL against MCF-7 and A-549 cells, respectively. 12-Hydroxyerosone (**5**) and isoelliptol (**6**) were less active. With this small data set, no definitive structure−activity relationships can be drawn; however, the following observations can be noted. The change from the 3,4-isopropenyldihydrofuran of **4** to the 2,3-furan ring of **5** caused a substantial decrease in potency. With its closed benzopyran ring, compound **5** was 2-fold less potent than the structurally related phenyl-substituted furanochromenone **6**. Interestingly, the change from the dimethoxy substitution of **6** to the methylenedioxy substitution of dehydroneotenone abolished activity. Finally, the four pterocarpans (**1**, **2**, 2-hydroxypterocarpin, neodulin) and the coumarin **7** showed little or no activity.

Experimental Section

General Experimental Procedures. A DIP-1000 digital polarimeter (JASCO, cell length 10 mm) was used for optical rotations. ^{1}H and ^{13}C NMR spectra were taken on a Varian Gemini 2000 300 MHz NMR spectrometer with TMS as internal reference in $CDCl_3$ and CD_3OD unless otherwise indicated. HRSIMS were obtained using a Hitachi M-4100H mass spectrometer. HPLC separations were conducted with a Shimadzu LC-610 instrument using an ODS column (YMC) and MeOH−H_2O (7:3) as solvent system.

Plant Material. The roots of *Neorautanenia mitis* (A. Rich) Verdcourt were collected in the Rukwa region of Tanzania in November 1993 by Dr. H. Schmidt et al. with the support of the Missouri Botanical Garden and The Institute of Traditional Medicine, Muhumbili University College of Health Sciences, Tanzania. A voucher specimen is housed in the Botany Department of the Museum of Natural History, Smithsonian Institution, Washington, D.C.

Isolation of Compounds from *N. mitis*. Air-dried roots (1.3 kg) were ground and extracted with MeOH−CH_2Cl_2 (1:1) to give a crude extract (52 g) after evaporation of the solvent. The dark red solid extract (10 g) was dissolved in MeOH, and the hexane-soluble components were separated. After adding EtOAc to the MeOH solution, the resulting insoluble material (0.73 g) was removed by filtration. The filtrate was evaporated in vacuo, and the concentrate (8.0 g) was fractionated by silica gel column chromatography (hexane−EtOAc stepwise gradient starting with 2:1 ratio) to give eight fractions on the basis of TLC behavior. The first fraction gave a solid that was recrystallized from hexane−EtOAc (10:1) to give neodulin (15 mg). Pachyrrhizine (**7**, 110 mg) was obtained from the third fraction by repeated recrystallization from hexane−EtOAc (10:1). Rotenone (**3**, 115 mg), 2-hydroxypterocarpin (2.6 mg), dehydroneotenone (2.0 mg), and 12a-hydroxyrotenone (**4**, 27 mg) were separated from the fourth fraction by sequential use of silica gel column chromatography and HPLC. 12a-Hydroxyerosone (**5**, 30 mg) and isoelliptol (**6**, 4.5 mg) were obtained from the fifth fraction by use of silica gel column chromatography and HPLC. Rautandiol A (**1**, 8.0 mg) and rautandiol B (**2**, 6.0 mg) were obtained from the sixth fraction using HPLC and finally silica gel column chromatography ($CHCl_3$−MeOH, 25:1).

Rautandiol A (1): colorless fine needles; mp 146−147 °C; $[\alpha]^{23}_D$ −201.0° (*c* 0.8, MeOH); ^{1}H NMR (CD_3OD), Table 1; ^{13}C NMR (CD_3OD) δ 133.2 (d, C-1), 114.4 (s, C-1a), 115.4 (s, C-2), 155.6 (s, C-3), 105.3 (d, C-4), 156.4 (s, C-4a), 67.7 (t, C-6), 41.1 (d, C-6a), 126.0 (d, C-7), 119.5 (s, C-7a), 108.7 (d, C-8), 159.9 (s, C-9), 98.8 (d, C-10), 162.0 (s, C-10a), 79.9 (d, C-11a), 31.6 (t, C-1′), 70.6 (d, C-2′), 78.4 (s, C-3′), 26.0 (q, C-4′), 21.0 (q, C-5′); HRSIMS *m*/*z* 341.1390 $[M + H]^+$ (calcd for $C_{20}H_{21}O_5$, 341.1388). *p*-Bromobenzoate of **1** (**1a**): mp 158−159 °C; ^{1}H NMR ($CDCl_3$) δ 7.25 (1H, s, H-1), 6.44 (1H, s, H-4), 4.27 (1H, dd, J = 9.8 Hz, 3.9, H-6eq), 3.64 (1H, dd, J = 10.7 Hz, 9.8, H-6ax), 3.57−3.67 (1H, m, H-6a), 7.27 (1H, d, J = 8.1 Hz, H-7), 6.74 (1H, dd, J = 8.1 Hz, 1.8, H-8), 6.70 (1H, d, J = 1.8 Hz, H-10), 5.57 (1H, d J = 6.3 Hz, H-11a), 3.07 (1H, dd, J = 16.5 Hz, 4.5, H-1′), 2.77 (1H, dd, J = 16.5 Hz, 6.5, H-1′), 3.86−3.77 (1H, m, H-2′), 1.35, 1.35 (3H each, s, H-4′,5′), 8.04 (2H, d, J = 8.9 Hz, benzoate), 7.65 (2H, d, J = 8.9 Hz, benzoate).

Rautandiol B (2): colorless needles; mp 215−216 °C; $[\alpha]^{23}_D$ −194.5° (*c* 0.6, MeOH); ^{1}H NMR (CD_3OD), Table 1; ^{13}C NMR δ

127.7 (d, C-1), 113.6 (s, C-1a), 122.5 (s, C-2), 162.7 (s, C-3), 98.6 (d, C-4), 157.4 (s, C-4a), 67.8 (t, C-6), 40.9 (d, C-6a), 126.0 (d, C-7), 119.6 (s, C-7a), 108.7 (d, C-8), 159.8 (s, C-9), 98.8 (d, C-10), 162.0 (s, C-10a), 80.4 (d, C-11a), 30.8 (t, C-1′), 91.4 (d, C-2′), 72.5 (s, C-3′), 25.4 (q, C-4′), 25.2 (q, C-5′); HRSIMS *m*/*z* 341.1389 $[M + H]^+$ (calcd for $C_{20}H_{21}O_5$ 341.1388). *p*-Bromobenzoate of **2** (**2a**): mp 189−190 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (1H, s, H-1), 6.39 (1H, s, H-4), 4.27 (1H, dd, J = 10.5 Hz, 4.4, H-6eq), 3.68 (1H, dd, J = 10.5 Hz, 10.5, H-6ax), 3.63−3.56 (1H, m, H-6a), 7.27 (1H, d, J = 8.1 Hz, H-7), 6.74 (1H, dd, J = 8.1 Hz, 2.0, H-8), 6.70 (1H, d, J = 2.0 Hz, H-10), 5.57 (1H, d, J = 6.3 Hz, H-11a), 3.13 (2H, d, J = 9.0 Hz, H-1′), 4.66 (1H, t, J = 9.0 Hz, H-2′), 1.33, 1.21 (3H each, s, H-4′,5′), 8.04 (2H, d, J = 8.9 Hz, benzoate), 7.56 (2H, d, J = 8.9 Hz, benzoate).

Cytotoxicity Assay. All stock cultures were grown in T-25 flasks (5 mL of RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin). Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well with test compounds from DMSO diluted stock. After 3 days in culture, cells attached to the plastic substratum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The IC_{50} is the concentration of test compounds that reduced cell growth by 50% over a 3-day assay period.

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