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Flavonoids from *Tephrosia aequilata*

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Abstract

From the roots of the plant *Tephrosia aequilata* Baker, five flavonoids were isolated of which, 3,4:8,9-dimethylenedioxypterocarpan is reported for the first time. Its structure and those of the already known flavonoids were established by physical and spectroscopic analysis. Application of 2D NMR techniques was useful for complete characterization of the new pterocarpan as well as the other known flavonoids.

Keywords: *Tephrosia aequilata*; Papilionaceae; Roots; Parasitic protozoa; Antimicrobial activity; 3,4:8,9-Dimethylenedioxypterocarpan; Praecansone A; Praecansone B; *Z*-Praecansone A; Demethylpraecansone B

1. Introduction

Tephrosia (Papilionaceae) is a large genus of perennial woody shrubs, which are well distributed in the tropical and sub-tropical regions of the world (Gillet et al., 1971). Between 300 and 400 species are known (Willis, 1973), of which 35 occur in India, 30 are native to South America, 70 are found in South Africa and 50 in equatorial Africa of which 30 are found in Kenya (Chadra, 1976; Allen and Allen, 1981; Beentje, 1994).

Some of the species have been used in herbal remedies, insecticides and rat, fish and human poisons by the various indigenous people of Kenya (Gillet et al., 1971; Watt and Breyer-Brandwijk, 1962). Phytochemical studies have been carried out on the roots of some *Tephrosia* species. For example, the roots of *T. emoroides* A. Rich., yielded 4″,5″-dihydro-5-methoxy-5″-*iso*-phenylfurano-[2″,3″,7,8]-flavanone which showed insect antifeedant activity against the larvae of stalk borer, *Chillo partellus* (Machocho et al., 1995). The roots of *T. hildebrandtii* Vatke yielded a pterocarpan, hildecarpin which exhibited antifeedant activity against the legume pod-borer, *Maruca testulalis* (Lwande et al., 1986). *T. interrupta* Engl. and *T. linearis* (Willd.) Pers. both yielded various rotenoids including deguelin and rotenone (Were, 1988). There has been no phytochemical investigation of *T. aequilata*. The roots of *T. aequilata* are used to treat venereal diseases and the leaves to relieve abdominal pains (Kokwaro, 1993; Gillet et al., 1971).

In the present investigation, five flavonoids were isolated from the petrol (bp 40–60 °C) extract of the roots of *T. aequilata*. The pterocarpan, 3,4:8,9-dimethylenedioxypterocarpan (**1**) is reported for the first time. The β-oxygenated chalcones, praecansone A (**2**) and praecansone B (**3**) have been reported earlier from *T. praecans* Brummitt (Camele et al., 1980), *T. procumbens* (Venkataratnam et al., 1987) and *T. pumila* Lam. (Dagne et al., 1988; Yenesew et al., 1989). The *Z*-isomer of praecansone A (**4**) is also reported as a plant metabolite while demethylpraecansone A (**5**) was reported previously from *Lonchocarpus costaricensis* Pittier (Waterman and Mahmoud, 1985).

2. Results and discussion

The five flavonoids were isolated from the petrol (bp 40–60 °C) extract of the roots by a combination of chromatographic techniques followed by crystallization.

The ^{1}H NMR spectral data of **1** (Table 1) suggested a pterocarpan structure due to the splitting pattern of the protons of the heterocyclic ring B and the bridging protons of B and C rings (Màximo and Lourenço, 1998; Machocho et al., 1995; Pachler and Underwood, 1967). The shifts appeared at δ 5.43 *d*, 4.23 *dd*, 3.64 *t* and 3.45

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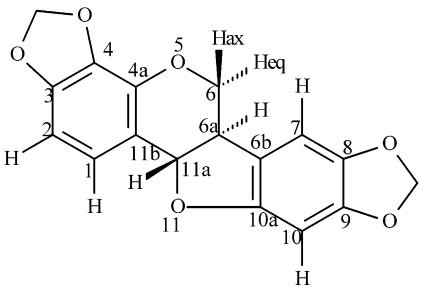

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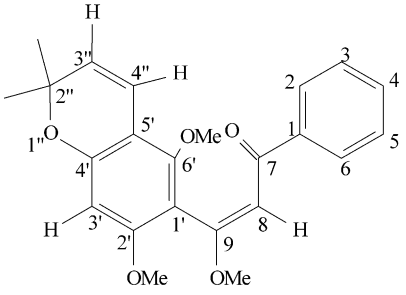

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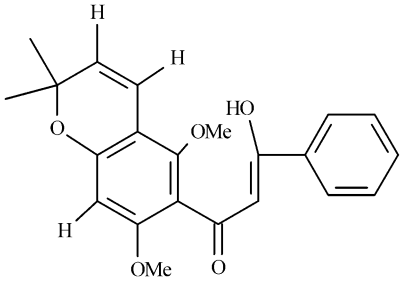

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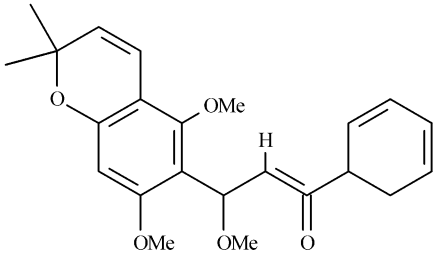

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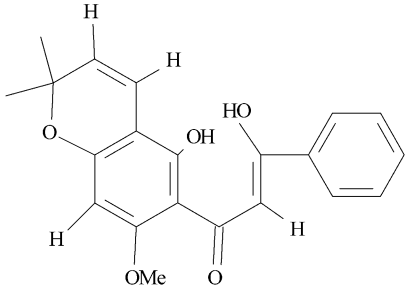

5

ddd, which were assigned to H-11a, H-6ax, H-6eq and H-6a, respectively. The spectrum exhibited four aromatic protons in which the two adjacent protons in positions 1 and 2 appeared at δ 6.96 *d* and 6.55 *d*, respectively, with *J* values of 8.1 Hz on the tetra-substituted ring A. The other two *para*-oriented signal at δ 6.66 *s* and 6.37 *s* were assigned to protons of positions 7 and 10, respectively, on ring D. The assignment of these sets of aromatic protons was supported by the ROESY spectrum, whereby H-1 showed spatial contours with H-11a and H-2, and H-7 with H-6ax, H-6eq and H-6a. These assignments were supported by 3-bond correlation in the HMBC spectrum. The two sets of aromatic protons suggested the placement of the two methylenedioxy groups at δ 5.84 (2*d*, $J = 1.5$ Hz) and 5.93 *s* at positions 3,4 and 8,9, respectively. Additionally, the two pairs of methylenedioxy protons showed 3-bond correlation in the HMBC spectrum with their respective aromatic carbon atoms.

The ^{13}C NMR spectral data of **1** (Table 1) indicated 17 carbon atoms and was in agreement with the proposed structure. The HMQC and HMBC spectra confirmed structure **1** for the new pterocarpan. The HMQC spectrum provided the assignment of the protonated aromatic carbons as follows: δ 123.5 (C-1), 102.1 (C-2), 104.1 (C-7) and 93.3 (C-10). The protonated carbons of the heterocyclic rings were observed at δ 77.6 (C-11a), 66.0 (C-6) and 39.6 (C-6a), which compared closely with literature values (Màximo and Lourenço, 1998; Lwande et al., 1986). The methylenedioxy carbons appeared at δ 101.2 and 100.7. The quaternary aromatic carbons were assigned with the help of the HMBC spectrum. The

Table 1
The ^{1}H NMR, HMQC and HMBC spectral data for compound **1**

Proton	^{1}H NMR (J in Hz)	Correlated C-atom	
		HMQC	HMBC
1	6.96 *d* (8.1)	123.5 *d*	C-11a, C-4a, C-3
2	6.55 *d* (8.1)	102.1 *d*	C-11b, C-4
		143.0 *s* (C-3)	
		143.3 *s* (C-4)	
		166.8 *s* (C-4a)	
6 (eq)	4.23 *dd* (10.8, 5.1)	66.0 *t*	C-11a, C-6b, C-4a
6 (ax)	3.64 *t* (10.8)	66.0 *t*	C-11a, C-6b, C-4a
6a	3.45 *ddd* (10.8, 6.9, 5.1)	39.6 *d*	C-11b, C-10a
		117.0 *s* (C-6b)	
7	6.66 *s*	104.1 *d*	C-10a, C-9, C-6a
		148.3 *s* (C-8)	
		148.3 *s* (C-9)	
10	6.37 *s*	93.3 *d*	C-8, C-6b
		160.0 *s* (C-10a)	
11a	5.43 *d* (6.9)	77.6 *d*	C-10a, C-6b, C-4a
		114.8 *s* (C-11b)	
3,4-OCH_2O-	5.84 *d* (1.5)	100.7 *t*	C-3, C-4
8,9-OCH_2O-	5.93 *s*	101.2 *t*	C-9, C-8

The carbon multiplicities were determined by DEPT data.

mass spectrum of **1** showed an $[M]^+$ at m/z 312 for $C_{17}H_{12}O_6$ thus confirming the above deductions based on NMR data analysis.

The ^{1}H NMR and ^{13}C NMR spectra of **2** are similar to those of praecansone A which had earlier been reported from *T. praecans* as praecansone A (Camele et al., 1980), and later the structure was revised by Dagne et al. (1988). Compounds **2** and **4** had different physical and chromatographic properties but had closely related UV and NMR spectral data. The ^{1}H NMR spectra of **2** and **4** were similar with minor variations. Each compound had three sets of methoxyl groups, two isolated protons, and unsubstituted benzene ring and a dimethyl chromene ring as the prenyl substitution. In the ^{1}H NMR of **2** the olefinic signal at δ 6.44 for H-8 (H-α) showed ROESY contours with H-2 or H-6 of the unsubstituted benzene ring and one of the methoxyl groups at δ 3.86, which was assigned to position 9. This implied that this particular methoxyl group was in the *trans*-orientation with respect to the hydrogen. One of the other methoxyl groups at δ 3.72 showed a spatial correlation with H-4″ signal at δ 6.50 and this was assigned to position 6′. The remaining methoxyl group at δ 3.66 was assigned to position 2′ based on the spatial correlation with the lone aromatic proton of position 3′ at δ 6.19. The ^{13}C NMR spectrum of **2** is identical to that of Praecansone A occurring as the *E*-isomer especially the ^{13}C NMR peak at δ 101.2 (C-8) as reported by Kiuchi et al. (1990). No spatial correlation in the ROESY spectra was observed between the olefinic proton at position 8 (H-α) with the methoxyl group at position 9 in **4**. The ^{1}H NMR spectra of **4** showed the signal of H-8 (H-α) appearing at δ 6.03 which seems to have moved upfield compared to a similar proton in **2** while in the ^{13}C NMR, the C-8 signal has moved down field to δ 104.8. It therefore appears that **4** is the *Z*-isomer of praecansone A (**2**) when compared to the ^{1}H NMR and ^{13}C NMR data of the β-methoxychalcones (Kiuchi et al., 1990). The EIMS spectra of **2** and **4** had $[M]^+$ at m/z 380 for $C_{23}H_{24}O_5$. The base peaks at m/z 349 represented lose of methoxyl groups. A peak at m/z 365 in both compounds suggested loses of methyl groups from the molecular ions.

In vitro biological activity tests against parasitic protozoa were performed on the isolated flavonoids. Compounds **3** and **5** showed low activity against *Trypanosoma brucei rhodensiense* (strain STIB 900, stage trypomastigotes) with IC_{50} 5.9 and 5.1 μg/ml, respectively, and *Trypanosoma cruzi* (strain Tulahuen C4, stage trypomastigotes) with IC_{50} 7.6 and 6.0 μg/ml, respectively. The compounds exhibited no cytotoxicity towards L-6 cells and macrophages but showed considerable activity against *Leishmania donovani* (strain MHOM-ET-67, stage amastigotes) with at IC_{50} values 17.2 and 9.0 μg/ml, respectively. Compounds **2** and **4** exhibited no activity against any of the parasitic protozoa or against *Plasmodium falciparum* (strain K1 and NF54, stages IEF).

Compounds **1**–**3** exhibited low activity against gram-positive bacteria, *Bacillus subtilis* and *Micrococcus lutea* and **4** and **5** even less activity ($\leqslant$8 mm). Compound **3** showed an inhibition zone of 11 and 13 mm against *B. subtilis* and *M. lutea,* respectively. The crude petrol (bp 40–60 °C) extract was inactive against the gram-negative bacteria, *Escherichia coli* and *Pseudomonas aureginosa* when tested at 100 μg/disk while **5** showed some activity (inhibition zone = 10 mm) against these bacteria. The other compounds showed slight or no activity against the gram-negative bacteria. The compounds were not active against the fungus, *Aspergillus niger* and the yeast, *Saccharomyces cerevisiae*. The antibacterial activities of these flavonoids were much lower than those observed for the standard antibiotics especially cotrimoxazole, streptomycin, kanamycin, gentamycin and chloramphenicol.

3. Experimental

3.1. General experimental procedures

Mps were uncorrected IR spectra: Perkin-Elmer 598 FTIR series spectrometer in KBr pellet. UV: Perkin-Elmer lambda 16 UV/vis spectrometer in MeOH. EIMS: Hewlett Packard 5989 A mass spectrometer at 70 eV with direct probe insert at 120–140 °C. NMR:

Varian VXR 500; $CDCl_3$ at 500 MHz for ^1H NMR and 75 MHz for ^{13}C NMR with TMS as int. standard and the chemical shifts reported in δ (ppm) units relative to TMS signal and coupling constants (J) in Hz. Silica gel SDS chromagel 60 A CC (6–35 μm) was used for VLC, and silica gel 60 F_{254} (Macherey-Nagel) for analyt. (0.25 mm) and prep. (0.25 mm) TLC. Spots on chromatograms were detected under UV light (254 and 365 nm) and by spraying with 25% aqueous H_2SO_4.

3.2. Plant material

The roots of *T. aequilata* Baker were collected at the summit of Nzaui Hills in Makueni District, Kenya in December, 1999. The sample was authenticated by Mr. Simon Mathenge, Botany Department, University of Nairobi, Kenya. A voucher specimen (SM/PKT/02/99) has been deposited in the herbarium, Nairobi University, Nairobi.

3.3. Extraction and isolation

Air-dried roots (1.64 kg) were extracted with petrol (bp 40–60 °C). After evaporation of solvents, a yellow paste (11.3 g) was obtained and the dried extract was chromatographed on a silica gel by VLC and eluted with *n*-hexane–EtOAc mixtures from the ratio of 9:1 to 1:1 to obtain fractions A to D. When fraction A was repeatedly recrystalized in Me_2CO, **5** (30.0 mg) was obtained as yellow needle-like crystals. fraction B was rechromatographed on silica gel and eluted with petrol (bp 60–80 °C): EtOAc (9:1) which on recrystallization in Me_2CO yielded **1** (19.1 mg). Fraction C from the VLC column was twice subjected to silica gel prep. TLC eluting with $CHCl_3$:EtOAc (1:1) and $CHCl_3$ (100%), respectively, to yield **3** (11.7 mg). Fraction D was rechromatographed on silica gel by VLC with petrol (bp 60–80 °C):EtOAc (4:1) as eluant and was later subjected to silica gel prep. TLC with $CHCl_3$: EtOAc (1:1) as eluant to yield **2** (22.7 mg) and **4** (17.2 mg).

3.4. 3,4-Dimethylenedioxypterocarpan (*1*)

White crystals, mp 154–156 °C. IR ν_{max}^{KBr} cm^{-1}: 2940, 1610, 1480, 1460, 1360, 1150, 1050, 1030, 1010, 930, 830. UV λ_{max}^{MeOH} nm: 209, 239, 304. ^1H NMR spectral data (500 MHz, $CDCl_3$) and ^{13}C NMR spectral data (75 MHz, $CDCl_3$): cf. Table 1. EIMS (probe) 70 eV, m/z (rel. int.): 312 $[M]^+$ (100), 295 (13), 225 (6), 175 (16), 165 (10), 162 (26), 149 (21), 139 (11), 125 (8), 111 (13), 97 (18), 85 (17), 83 (20), 81 (15), 71 (28), 69 (31), 57 (46), 55 (31).

3.5. Praecansone A (*2*)

Yellow oil (17.9 mg), IR ν_{max}^{KBr} cm^{-1}: 2940, 1610, 1480, 1460, 1360, 1150, 1050, 1030, 1010, 930,830. λ_{max}^{MeOH} nm: 209, 239, 304. ^1H NMR spectral data (500 MHz, $CDCl_3$) and ^{13}C NMR spectral data (75 MHz, $CDCl_3$): δ 7.83 (2H, *m*, H-2, H-6), 7.42 (1H, *m*, H-4), 7.37 (2H, *m*, H-3, H-5), 6.50 (1H, *d*, $J=10$ Hz, H-4″), 6.44 (1H, *s*, H-8), 6.19 (1H, *s*, H-3′), 5.43 (1H, *d*, $J=10$ Hz, H-3″), 3.86 (3H, *s*, 9-OMe), 3.72 (3H, *s*, 6′-OMe), 3.66 (3H, *s*, 2′-OMe), 1.42 (6H, *s*, 2″-Me_2). ^{13}C xNMR spectral data (75 MHz, $CDCl_3$): 190.2 (C-7), 166.1 (C-9), 157.7 (C-4′), 154.5 (C-6′), 155.6 (C-2′), 139.7 (C-1), 131.6 (C-4), 128.0 (C-2, C-6), 127.7 (C-3, C-5), 127.0 (C-4″), 116.8 (C-3″), 111.9 (C-5′), 101.2 (C-8), 96.0 (C-3′), 76.5 (C-2″), 62.2 (2′-OMe), 56.2 (9-OMe), 55.8 (6′-OMe), 28.0 (2″-Me_2). EIMS (probe) 70 eV, m/z (rel. int.): 380 $[M]^+$ (6), 365 (20), 349 (100), 335 (8), 319 (18), 245 (5), 217 (4), 167 (14), 105 (22), 77 (17).

3.6. Praecansone B (*3*)

Yellow oil (17.9 mg), IR ν_{max}^{KBr} cm^{-1}: 2980 and 3009, 1670, 1610, 1560, 1480, 1370, 1200, 1150, 1130. UV λ_{max}^{MeOH} nm: 274, 309. ^1H NMR spectral data (500 MHz, $CDCl_3$) and ^{13}C NMR spectral data (75 MHz, $CDCl_3$): δ 7.90 (2H, *m*, H-2, H-6), 7.50 (1H, *m*, H-4), 7.40 (2H, *m*, H-3, H-5), 6.50 (1H, *d*, $J=10$ Hz, H-4″), 6.47 (1H, *s*, H-8), 6.23 (1H, *s*, H-3′), 5.50 (1H, *d*, $J=10$ Hz, H-3″), 3.78 (3H, *s*, 6′-OMe), 3.76 (3H, *s*, 2′-OMe), 1.43 (6H, *s*, 2″-Me_2). ^{13}C NMR spectral data (75 MHz, $CDCl_3$): 188.0 (C-9), 182.0 (C-7), 158.2 (C-4′), 156.2 (C-2′), 155.0 (C-6′), 134.0 (C-1), 132.1 (C-4), 128.5 (C-2, C-6), 127.7 (C-4″), 127.0 (C-3, C-5), 116.5 (C-3″), 114.0 (C-5′), 100.5 (C-8), 96.2 (C-3′), 76.4 (C-2″), 63.2 (6′-OMe), 56.1 (2′-OMe), 28.0 (2″-Me_2). EIMS (probe) 70 eV, m/z (rel. int.): 366 $[M]^+$ (16), 351 (100), 335 (95), 321 (4), 305 (11), 247 (16), 231 (9), 217 (17), 205 (52,) , 190 (7), 175 (7), 160 (7), 105 (25), 91 (6), 77 (29), 69 (14), 51 (6).

3.7. cis-Praecansone A (*4*)

Yellow oil (17.9 mg), Found $[M]^+$ 380.1537; $C_{23}H_{24}O_5$ Calc. for 380.1624. IR ν_{max}^{KBr} cm^{-1}: 1660. UV λ_{max}^{MeOH} nm: 284. ^1H NMR spectral data (500 MHz, $CDCl_3$): δ 7.89 (2H, *m*, H-2, H-6), 7.43 (1H, *m*, H-4), 7.38 (2H, *m*, H-3, H-5), 6.50 (1H, *d*, $J=10$ Hz, H-4″), 6.22 (1H, *s*, H-3′), 6.03 (1H, *s*, H-8), 5.52 (1H, *d*, $J=10$ Hz, H-3″), 3.78 (3H, *s*, 9-OMe), 3.70 (3H, *s*, 6′-OMe), 3.65 (3H, *s*, 2′-OMe), 1.44 (6H, *s*, 2″-Me_2). ^{13}C NMR spectral data (75 MHz, $CDCl_3$): 189.0 (C-7), 164.0 (C-9), 158.3 (C-4′), 156.0 (C-6′), 155.0 (C-2′), 140.0 (C-1), 131.5 (C-4), 128.1 (C-2, C-6), 127.8 (C-3, C-5), 127.6 (C-4″), 116.1 (C-3″), 110.0 (C-5′), 104.8 (C-8), 95.7 (C-3′), 76.4 (C-2″), 62.2 (2′-OMe), 57.3 (9-OMe), 55.9 (6′-OMe), 28.1 (2″-Me_2). EIMS (probe) 70 eV, m/z (rel. int.): 380 $[M]^+$ (11), 365 (19), 349 (100), 335 (8), 319 (16), 245 (4), 217 (3), 167 (9), 105 (12), 77 (9).

3.8. Demethylpraecansone B (5)

Yellow needle-like crystals from *n*-hexane-EtOAc (9:1), (17.9 mg), mp 126–129 °C, lit. (Waterman and Mahmoud, 1985). Found $[M]^+$ 352.1317; $C_{21}H_{20}O_5$ Calc. for 352.1311. ν_{max}^{KBr} cm^{-1}: 1640, 1580, 1280. UV λ_{max}^{MeOH} nm: 279, 369. ^1H NMR spectral data (500 MHz, $CDCl_3$): δ 7.86–7.91 (2H, *m*, H-2, H-6), 7.45–7.50 (3H, *m*, H-3, H-4, H-5), 7.32 (1H, *s*, H-8), 6.69 (1H, *d*, $J=10$ Hz, H-4″), 5.95 (1H, *s*, H-3′), 5.47 (1H, *d*, $J=10$ Hz, H-3″), 3.92 (3H, *s*, 2′-OMe), 1.46 (6H, *s*, 2″-Me_2). ^{13}C NMR spectral data (75 MHz, $CDCl_3$): 194.0 (C-9), 175.8 (C-7), 162.0 (C-4′), 160.5 (C-2′), 159.9 (C-6′), 133.5 (C-1), 131.6 (C-4), 128.7 (C-2, C-6), 128.1 (C-3, C-5), 126.6 (C-4″), 116.1 (C-3″), 104.0 (C-3′), 103.0 (C-1′), 98.2 (C-8), 91.8 (C-5′), 76.4 (C-2″), 55.9 (2′-OMe), 28.4 (2″-Me_2). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 352 $[M]^+$ (17), 337 (40), 319 (3), 232 (3), 217 (100), 202 (6), 191 (24), 105 (20), 77 (25), 69 (5), 51(4).

3.9. Parasitic assay

The assays to determine activity of the flavonoids for *T. brucei rhodensiense*, *T. cruzi* and *Leishmania donovani* were carried out according to the method of Räz et al. (1997) and Baltz et al. (1985). The antiplasmodium activity was determined as described by Ridley et al. (1996).

3.10. Antimicrobial assay

The bioassay for antimicrobial activity was carried out by agar diffusion assay method as described by Barry et al. (1979).

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