FLAVONOID CONSTITUENTS OF THE MATURE FRUIT OF *TETRAPLEURA TETRAPTERA* SCHUM. *ET* THONN.)

T.C. Fleischer[1], G. Komlaga[1], A.Y. Mensah[1], M.L.K. Mensah[1], E. Wood[2] I.K. Sawer[3] and A.I. Grey[4]

[1]*Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST., Kumasi, Ghana*

[2]*Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST., Kumasi, Ghana*

[3]*Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST., Kumasi, Ghana*

[4]*Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, Scotland*

ABSTRACT

Phytochemical investigation of the ethanolic extract of the mature fruit of Tetrapheura tetraptera led to the isolation of three flavonoid constituents namely 2', 4, 4'-trihydroxychalcone, 2', 3, 4, 4'-tetrahydroxychalcone and 4', 5, 7-trihydroxyflavanone. The structures were established from 1D and 2D NMR experiments. This is the first isolation of flavonoid constituents in the genus Tetrapleura.

Keywords: *Tetrapheura tetraptera, flavonoid, chalcone, flavanone, 1D and 2D NMR.*

INTRODUCTION

Tetraphera tetraptera (Schum. *Et* Thonn.) (Mi-mosaceae) is a tree that grows in the tropical deciduous forest of West Africa, extending from Senegal to West Cameroon; and also found in Sudan, Uganda and Zaire (Burkill, 1995). In West Africa, the fruit is widely used in traditional remedies for the treatment of several conditions including convulsion, gastric ulcer, rheumatism, fevers, whitlow, skin rashes, smallpox, malaria and dysentery (Dalziel, 1995, Irvine, 1961, Abbiw, 1990). Previous phytochemical studies revealed the presence of triperpene saponins of the oleanane type (Adesina and Sofowora, 1979; Mailland *et al,* 1992 Ngassapa *et al.,* 1993). The occurrence of flavonoids and other classes of secondary metabolites have been reported only in the stem bark (El-izzi *et al.,* 1990). Recently we have taken keen interest in the chemistry and pharmacology of the fruit of *Tetrapheura tetraptera* because of the reports that it is used to manage diabetes and cardiovascular diseases like hypertension and stroke in some traditional societies of Ghana (Amoako-Atta B., Director - CBUD, KNUST, personal communication, 2001). In this report we present the isolation and characterization of three flavonoid constituents from the ethanolic extract of the fruit.

MATERIALS AND METHODS

General

1D and 2D NMR spectra were obtained on a Bruker AMX - 400 spectrometer operating at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR). The NMR experiments performed included proton nuclear magnetic resonance (^{1}H-NMR), ^{13}C-nuclear magnetic resonance (^{13}C-NMR), Homonuclear Correlation Spectroscopy (^{1}H-^{1}H COSY), Heteronuclear Multiple Bond Connectivity (HMBC) and Nuclear Overhauser Effect (NOESY). The *J*-modulated ^{13}C-spectra were acquired with a relaxation time (d_1) of 6 sec. The HMBC spectra were optimised for long range $J_{H\text{-}C}$ of 7 Hz (d_6 = 0.007 sec). The ^{1}H-NMR and ^{13}C-NMR chemical shifts were referenced to solvent signals at $\delta_{C/H}$ 7.27/77.0 ($CDCI_3$) and 4.78/49.0 (CD_3OD) relative to TMS.

Column chromatography was carried out using Merck Silica gel 40. Preparative TLC was carried out using Merck Silica gel 60 PF_{254} on glass plates (20 X 20 cm) at a thickness of 0.5mm. TLC was conducted on Merck Silica gel 60 F_{254} pre-coated on aluminium sheet.

Plant Material

Mature dried fruits of *Tetrapheura tetraptera* (Mimosaceae) were supplied in February 2002, by the Centre for Biodiversity Utilization and Development (CBUD), KNUST, a centre for promoting the valorisation of the fruits in indigenous societies in Ghana.

Extraction and Isolation

The fruits of *T. tetraptera* were cut into smaller pieces and dried in a hot air oven at 50^{o}C after which the dried material was milled to a coarse powder was soxhlet extracted with 70% EtOH for 12 hours. The extract was concentrated to a dark brown viscous liquid under reduced pressure using the Rotavapor. The liquid extract was further evaporated to a semisolid mass (Yield: 34.13%$^{w}/_{w}$). 100g of the extract was column chromatographed over silica gel, eluting sequentially in 100ml aliquots with petroleum ether followed by 20% and 50% ethyl acetate in petroleum ether, ethyl acetate, 20% and then 50% methanol in ethyl acetate. They were then bulked together, based on their TLC profiles, to give fractions A to F. Further column chromatographic fractionation of fraction D (7.44 g) on silica gel and preparative TLC using petroleum ether: ethyl acetate (6:4) as solvent system led to the isolation of 2', 4', 4-tetrahydroxychalcone (17.0mg) (**1**), and a mixture of 2', 4, 4'-trihy-droxychacone (**2**) and 4', 5, 7-trihydroxy-flavanone (8.5mg) (**3**).

RESULTS AND DISCUSSION

Compound **1** was obtained as yellow amorphous solid, melting point 210-218^{o}C (uncorrected). The ^{1}H NMR spectrum obtained in pyridine (Table 1) showed 6 aromatic signals at δ8.13 (*d j=8.9* Hz), δ7.80 (*d J*=1.9 Hz), δ7.36 (*dd J*=2.0 Hz, 8.2 Hz), δ 7.28 (*d J*=8.12 Hz), δ6.89 (*d J=2.32* Hz) and δ 6.78 (*dd J*=2.3 Hz, 8.8 Hz). It also showed two trans-coupling olefinic protons at δ8.24 (*d J*=15.2 Hz) and δ7.88 (*d J*=15.2 Hz), and a singlet at δ14.42 typical of extended hy-drogen bonding in 2'hydroxychalcones (Mabry *et al*., 1970; Fleischer *et al.,* 1997). The *J*-modulated ^{13}C NMR spectrum (Table 1) revelaed a carbonyl fucntion at δ 192.8, eight olefinic/aromatic methines at δ146.1, δ133.3, δ 123.4, δ118.3, δ 117.3, δ 117.0 δ 104.5, δ 109.6, four oxygenated aromatic carbon signals at δ148.3, δ151.5, δ168.0, and δ167.1, and two quaternary aromatic signals at δ128.0 and δ114.6. This suggested a molecular formula of $C_{15}H_{12}0_5$ for compound **1**. The COSY 90 spectrum (Fig. 1), revealed all the possible ^{1}H-^{1}H correlations. In the HMBC experiment, the *trans*-coupled olefinic protons at δ7.88 (H-α) and δ8.24 (H-*β*) showed ^{2}J and ^{3}J correlations respectively, with the carbonyl carbon (Fig. 2), confirming the characteristic propenone struc-ture in chalcones. H-*α* and H-*β* also showed ^{3}J and ^{2}J correlations respectively with the qua-ternary aromatic carbon at *δ*128.0 (C-1), confirming that the C-*β* of the propenone chain is linked to ring B. This carbon signal (C-1) further correlated (^{2}J) with the proton doublet at δ7.80 (H–2) and the doublet of doublet at δ7.36 (H-6). The long range correlations between 2'-

Table 1: ^{1}HNMR and ^{13}C NMR data for 1 and 2

Position	δ_H		δ_C	
	1	**2**	**1**	**2**
1			128.0	126.5
2	7.80 (*d*, *J=1.9* Hz)	7.43 (*d*, *J*=8.6 Hz)	117.0	130.6
3		6.76 (*d*, *J*=8.5 Hz)	148.3	116.0
4			151.5	159.8
5	7.28 (*d*, *J*=8.1 Hz)	6.76 (*d*, *J*=8.5 Hz)	117.3	116.0
6	7.36 (*dd*, *J*=1.96 Hz, 8.16 Hz)	7.43 (*d*, *J*=8.6 Hz)	123.4	130.6
α	8.24 (*d*, *J*=15.24 Hz)	7.64 (*d*, *J*=15.3 Hz)	118.3	117.2
β	7.88 (*d*, *J*=15.24 Hz)	7.33 (*d*, *J*=15.3 Hz)	146.1	144.6
C=O			192.8	192.1
1'			114.6	113.6
2'-OH	14.42 (*s*)		168.0	165.8
3'	6.89 (*d*, *J*=2.32 Hz)	6.26 (*d*, *J*=2.3 Hz)	104.5	103.1
4'			167.1	164.6
5'	6.78 (*dd*, *J*=2.28 Hz, 8.8 Hz)	6.32 (*dd*, *J*=2.3 Hz, 8.8 Hz)	109.6	108.3
6'	8.13 (*d*, *J=8.88* Hz)	7.71 (*d*, *J*=8.8 Hz)	133.3	131.9

Fig. 1: Structures of compounds 1-3. 2', 4, 4'- trihydroxychalcone(1), 2', 3, 4, 4'-tetrahyxychalcone (2) and 4', 5, 7-trihydroxyflavanone (3).

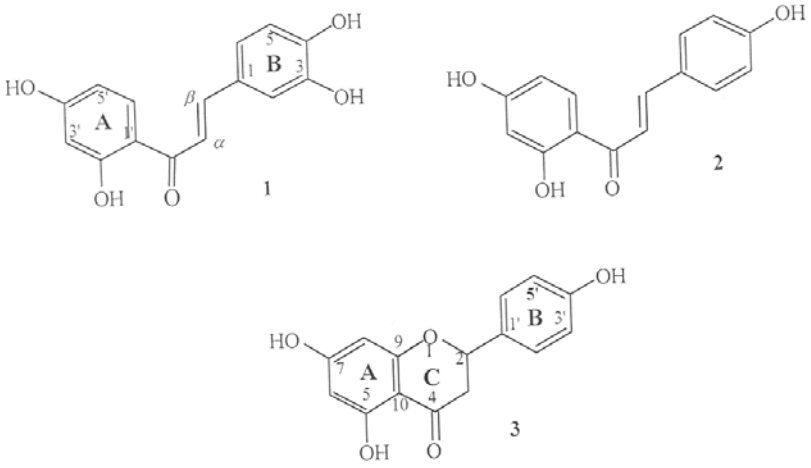


Table 2: ^{1}H-NMR and ^{13}C NMR data for 3

Pos.	δ_H	δ_C
2	5.2 (*dd, J*=3.0 Hz, 13.0 Hz)	79.1
3	2.62 (*dd, J*=3.0Hz, 17.2Hz [H-3eq])	43.0
	2.97 (*dd J*=13Hz, 17.2Hz [H-3ax])	
4		196.2
5		163.9
6	5.86 (*s*)	96.4
7		166.9
8	5.85 (*s*)	95.6
9		163.3
10		102.4
1'		129.4
2'	7.18 (*d, J*=8.6 Hz)	127.9
3'	6.76 (*d, J*=8.5 Hz)	115.6
4'		157.4
5'	6.76 (*d, J*=8.5 Hz)	115.6
6'	7.18 (*d, J*=8.6 Hz)	127.9

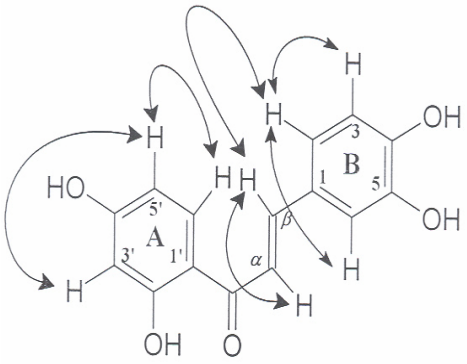
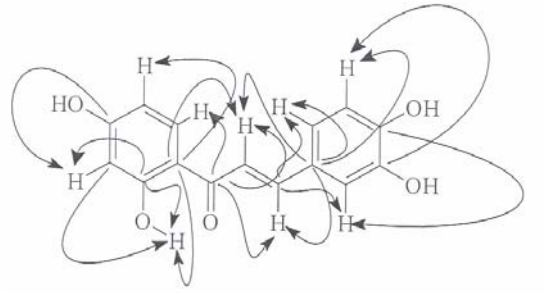


Fig. 2: Important ^{1}H-^{1}H COSY coupling in 1

OH (^{2}J) and H-6' (^{3}J) with C-2' confirmed the assignments. Other confirmatory long range correlations are shown in Fig. 2. The absence of relevant signals in the ^{1}H and ^{13}C NMR spectra suggested that the substituents on the three oxygenated quaternary aromatic carbons should be hydroxyl groups. Compound **1** was thus identified as the known 2', 3, 4, 4'-tetrahydroxy-chalcone which is trivially referred to as butein, previously isolated from various plant species including *Dalbergia odorigera*, (Cheng *et al*. 1998). The spectral data of **1** were in accordance with those published (Sritularak, *et al.,* 2002).

Fig. 3: Some significant long range (^{2}J and ^{3}J) correlations in the HMBC spectrum of 1



Compounds **2** and **3** were obtained as a mixture with **2** as the major component. The mixture occurred as a yellow amorphous solid, which melted at 240-251 (uncorrected). The spectral data of **2** were similar to that of **1**. The ^{1}H NMR spectrum in CD_3OD/CD_3OD (Table 1) showed the typical *trans*-coupled olefinic protons (H-α and H-β) of the propenone moiety of the chalcone at δ7.64 (*d, J*=15.3) and δ7.33 (*d, J*= 15.3Hz). It also exhibited two aromatic doublets at δ7.43 (*d, J*=8.6Hz), and δ6.76 (*d*, *J*=8.5Hz), each integrating for two protons, characteristic of an A_2X_2 system of *para*-distributed phenyl ring. It further revealed a doublet of doublets at δ6.32 (*dd, J*=2.3 Hz, 8.8 Hz) which *meta*-coupled with a doublet at δ6.26 (*d, J*=2.3 Hz) and *ortho*-coupled with another at δ7.71 (*d, J*=8.8 Hz) to give a trisubstituted phenyl ring. The ^{13}CNMR spectrum (Table 1) showed the presence of carbonyl carbon at δ192.1 (C=O), two olefinic carbon signals at δ144.6 (C-β) and δ117.2 (C-α), confirming the propenone submit of chalconesas in **1**. It also revealed three oxygenated aromatic carbons at δ159.8, δ164.6 and δ165.8 and other aromatic signals, giving the molecular formula of **2** as $C_{15}H_{12}O_4$. the COSY 90 spectrum revealed all the possible ^{1}H– ^{1}H correlations. The structure was established with the HMBC experiment. In this spectrum, the carbonyl carbon exhibited ^2J and ^3J correla-tions with the olefinic protons at δ7.33 (H-α) and δ7.64 (H-β) respectively. It also showed a ^3J correlation between the carbonyl carbon at β192.1 and the trisubstituted phenyl proton at δ7.71 (H-6') while the olefinic carbon at δ144.6 (C-β) showed ^3J correlations with the H-2/6 of the *p*-disubstituted benzene ring. The substi-tuents on the three oxygenated aromatic carbons of the phynyl rings must be OH groups, since there were no relevant signals in the ^{1}H NMR and ^{13}C NMR spectra. The expected extended hydrogen bonding signal for 2'-hydroxychal-cones in the ^{1}H NMR spectrum of **2**, was not evident due to deuterium exchange. Compound **2** was thus identified as 2', 4, 4'-trihydroxychal-cone (isoliquiritigenin) which was previously isolated from *Glycyrrhiza glabra* (Aida *et al*. 1990) and *Pterocarpus indicus* (Kusuma *et al*., 2004). A comparison of the spectral data of **2** with that of the literature (Veitch *et al*, 2003) confirmed the structure.

Compound **3**, the minor component of the mixture, give proton signals that were identical and sometimes overlapped with those of **2**. The ^{1}H NMR spectrum which was typical of a flavenone, showed two aromatic doublets centred at δ7.18 *d, J*=8.6Hz (H-2'/6') and δ6.76 *d, J*=8.5Hz (H-3'/5') characteristic of the *p*-disubstituted benzene ring system, an aromatic singlet at δ5.85 which integrated for two protons and assignable to H-6 and H-8 of ring a of a flavanone, and the typical flavanone ABX system at δ5.20 (*dd, J*=3.0 Hz, 13.0 Hz), δ2.62 (*dd, J*=3.0 Hz) and δ2.97, (*dd, J*=13.0 Hz, 17.2H.) (Mabry *et al*., 1970). The ^{13}C NMR spectrum (Table 2) showed signals for a carbonyl function at δ196.2, four oxygenated aromatic carbons at δ163.9, δ163.3, δ166.9 and δ157.4, two aromatic tertiary carbons at δ129.4 and δ102.4, an oxygenated methine carbon at δ79.1 and methylene signal at δ43.0, and six aromatic methines, providing for the molecular formula $C_{15}H_{12}O_5$. The flavanone struture was confirmed by the presence of the carbonyl sginal at δ196.2 assignable to C-4, the oxygenated methine and methylene signals at δ79.1 and δ43.0 allocated to C-2 and C-3 respectively in the ^{13}C NMR spectrum (Agrawal, 1989). Analysis of the 2D NMR experiments (^{1}H-^{1}H COSY, HMBC and ^{1}H-^{1}H NOESY spectra) supported the proton and carbon assignments. The other substituents on the phenyl ring subunits must be OH groups which were confirmed by the presence of oxygenated quaternary aromatic carbons at δ163.9 (C-5), δ166.9 (C-7) and δ157.4 (C-4'), and the lack of relevant signals in the ^{1}H NMR and ^{13}C-NMR spectra. In the ^{1}H NMR spectrum the expected extended H-bonding signal for 5-hydroxyflavanones did not show because of deuterium exchange. The structure of **3** was thus established as the known 4', 5, 7-trihydroxyflavanone (naringenin). The spectral data of compound **3** was consistent with those published (Shen *et al*., 1993).

The isolation of these flavonoids from the fruits of *Tetrapleura tetraptera* is noteworthy. This is the first report of the isolation of such consti-tuents from the genus *Tetrapleura,* although as a class of secondary metabolites, flavonoids have been reported to occur in some parts of *T. tetraptera* including the stem bark (El-izzi *et al.,* 1990). Secondly, the isolated flavonoids have demonstrated various biological activities. For example butein **1** has been shown to be a potent antioxidant and an anti-inflammatory agent (Cheng *et al*., 1998). Isoliquiritigenin **2** has shown vasorelaxant activity on the phynyleph-rine-precontracted rat aorta (Yu and Kuo, 1995), aldose reductase inhibiting activity (Aida *et al.,* 1990) and is also a potent anti-tumor-promoting and anti-inflammatory agent (Yamamoto *et al*., 1991). Naringenin **3** has been shown to lower plasma cholesterol *in vivo* (Lisa *et al*., 2001) and also have antioxidant and hypoglycaemic properties (Ali and El Kader, 2004). Thus the presence of these constituents in the fruit of *T. tetrapheura* could be said to have contributed to the hypoglycaemic effect of the extract observed in our laboratory (Komlaga *et al*.,

2004), and justifies the use of the fruit in the management of diabetes and hypertension in folklore medicine in Ghana.

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REFERENCES

Abbiw, D (1990). Useful plants of Ghana. West African use of wild and cultivated plants. Intermediate Technology Publications and the Royal Botanic Gardens, KEW. UK.52: 156.

Adesina, S.K. and Sofowora, E.A. (1979). The isolation of an anticonvulsant glycoside from the fruit of *Tetrapleura tetraptera. Planta Medica*. 36, 270-1.

Agrawal, P.K. (1992). NMR spectroscopy in the structure elucidation of oligosaccharides and glycosides. *Phytochemistry,* 31, 3307.

Aida K. Tawata N. Shindo . Onaya T, Sasaki H. Yamaguchi T, Chin M, Mitsuhashi H. (1990) Isoliquiritigenin: a newaldose reduc-tase inhibitor from *Glycyrrhiza radix. Planta Medica.* 56 254-8.

Aldrick Library of ^{13}C and ^{1}H FT NMR spectra (1992) 2, 915C.

Ali, M.M. and El Kader, M.A. (2004). The influence of naringenin of the oxidative state of rats with streptozotocin - induced acute hyperglycaemia. *Z. Naturforsch* 59, 726-733.

Burkill, H.M. (1995). The useful plants of West Tropical Africa. 2nd ed., royal Botanic Gardens, Kew, pp 263-265.

Cheng Z.J., Kuo S.C., Chan S.C., Ko F.N., Teng C.M. (1998). antioxidant properties of butein isolated from Dalbergia odorifera, *Biochim Biophys Acta*. 1392, 291-9.

Dalziel, J.M. (1955). The Useful Plants of West Tropical Africa, Crown Agents for Ovrseas Governments and Administrations. pp. 223-224.

El-izzi, A.: Benie, T.: Thieulant, M.L. and Duval, J. (1990). Inhibitory effects of saponins from *T. tetraptera* on the Luteini-zing Hormone release by cultural rat pituitary cells. *Planta Medica* 56: 359.

Fleischer, T.C., Waigh, R.D. and waterman, P.G. (1997). A novel retrodihydrochalcone from the stem bark of *Uvaria mocoli L., Phytochemistry*, 47 1387-1391.

Irvine, F.R. (1961). Woody plants of Ghana with special reference to their uses. Oxford University Press, London, 58.

Lisa J. Wilcox, Nica M. Borradaile, Linda E. de Dreu, and Murray W. Huff (2001). Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP, *Journal of Lipid Research,* 42 725-734.

Komlaga, G., Sarpong, K., Mensah, M.L.K., Mensah, A.Y., Wood, E., Sawer, I.K. and Fleischer, t.C. (2004). Hypoglycaemic activity of the alcoholic fruit extract of *Tetrapleura tetraptera* in normoglycaemic albino rats, (an oral presentation made at the Western African Network of Natural products Research Scientists, WANNPRES, 15-20 August, 2004, in Accra.

Kusuma I.W., Ogawa T., Itoh K. and Tachibana S. (2004). Isolation and Identification of an Antifungal Sesquiterpene Alcohol from Amboyna Wood *Pakistan Journal of Biological Sciences* 7 1735-1740.

Mabry, J.T, Markham, K.R. and Thomas, M.B. (1970). The systematic identification of flavonoids, *Springler*, New York Inc.

Maillard, M., Adewunmi, C.O. and Hostettmann K. (1992). A triterpene glycoside from the fruits of *T. tetraptera*. *Phytochemistry*, 31, 1321-1323.

Ngassapa, O., Beecher, C.W.W.; Pezzuto, J.M.; Farnsworth, N.R.; Henderson, T.O. and Boye, G.L. (1993). Isolation of echinocystic acid-3– sulphate a new triterpene, from *Tetrapluera tetraptera* and evaluation of the mutagenic potential of molluscicidal extracts and isolates. *J. Nat. Prod;* 56, 1872-1877.

Sritularak B.; Likhitwitayawuid K.; Conrad, J.; Kraus, W. (2002). Flavonoids from the roots of *millettia*

erythrocalyx. Phytochemistry; 61: 943-7.

Veitch, N.C., Sutton PS; Kite G.C. and Ireland H.E. (2003). Six new isoflavones and a 5-deoxyflavonol glycoside from the leaves of *Ateleiaherbert-smithii*. *J. Nat. Prod;* 66, 210-216.

Yamamoto S., Aizu E., Jiang H., Nakadate T., Kiyoto I., Wang J.C. and Kato R. (1991). The potent anti-tumor-promoting agent isoliquiritigenin. *Carcinogenesis*, 12 317-323.

Yu S.M. and Kuo S.C. (1995). Vasorelaxant effect of isoliquiritigenin, a novel soluble guanylate cyclase activator, in rat aorta. *Br J Pharmacol*. 114, 1587-94.