Phytochemistry, Vol. 23, No. 5, pp. 1202–1203, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

FLAVONOIDS OF *GEIGERIA*

P. C. COLEMAN*, D. J. J. POTGIETER, C. H. VAN ASWEGEN and N. M. J. VERMEULEN†

Departments of Chemistry and Biochemistry, University of Pretoria 0002, Republic of South Africa

(*Received* 4 *July* 1983)

Key Word Index—*Geigeria*; Compositae; 5,7,3',4'-tetrahydroxy-6-methoxyflavone; 5,7,4'-trihydroxy-6-methoxyflavone; ^{13}C NMR; respiratory inhibition.

Abstract—Chromatographic separation of the ethanol extract of *Geigeria burkei* resulted in the isolation and characterization of two known flavonoids, 5,7,3',4'-tetrahydroxy-6-methoxyflavone and 5,7,4'-trihydroxy-6-methoxyflavone. ^{13}C NMR data, as well as the respiratory inhibition and uncoupling of oxidative phosphorylation, is reported for the first time.

INTRODUCTION

Geigeria species are known to cause vomiting disease in stock [1]. Also present in these plants are sesquiterpene lactones [2]. The presence of two unidentified flavones have also previously been described in *G. aspera* [3]. The influence of flavonoids on biological systems has been reviewed [4].

In this paper we report the isolation and characterization of two known flavones 5,7,3',4'-tetrahydroxy-6-methoxyflavone (nepetin, eupafolin) [5, 6] and 5,7,4'-trihydroxy-6-methoxyflavone (dinatin, hispidulin) [7–9] from *Geigeria Burkei* Harv subsp. *burkei* var *zeyheri* Merxm. ^{13}C NMR data, as well as the effect of these flavones on respiration and uncoupling of oxidative phosphorylation, are reported for the first time.

RESULTS AND DISCUSSION

Compound **1**, mp 277–278° (lit. mp 280–282° [6]) and compound **2**, mp 297–298° (lit. 291–292° [9]) were identified as 5,7,3',4'-tetrahydroxy-6-methoxyflavone and 5,7,4'-trihydroxy-6-methoxyflavone respectively, mainly on the basis of ^{13}C NMR data. The ^{13}C NMR data is shown in Table 1 and the assignment of resonances to individual carbon atoms are discussed briefly.

Accurate mass determination and elemental analysis indicated a structural formula of $C_{16}H_{12}O_7$. The fact that 16 clear signals were obtained in pnd ^{13}C NMR spectrum indicated that there was no symmetry in the molecule and that the B ring was 3',4'-disubstituted. The ^{13}C NMR spectrum also showed the presence of one methoxyl group though it was found to resonate some 3 ppm to a higher field than normal. Comparison with published spectra [10] indicated that the B ring was a 3',4'-dihydroxyphenyl substituted ring and that the methoxyl group must be attached to ring A. From available data a 5- or 7-methoxy group was ruled out and literature data revealed a ^{13}C NMR shift pattern very similar to that reported for pectolinarigenin [6]. The compound also exhibited a singlet at δ131.2 and a methoxyl carbon resonance at 59.6 and on that basis it was concluded that the methoxy group must be attached to C-6, which identified compound **1**.

Compound **2** exhibited only 14 signals in the pnd ^{13}C NMR spectrum while elemental analysis and accurate mass determination indicated a structural formula of $C_{16}H_{11}O_6$. However the ^{13}C NMR showed that two peaks were far more intense than all the other peaks in the spectrum and this suggested that these peaks corresponded to two identical carbons (same molecular environment). The two intense ^{13}C NMR signals at δ115.8 and 128.2 indicated a symmetrically substituted B ring and this was confirmed to be a 4-hydroxyphenyl ring by comparison with published data [6].

The other ^{13}C NMR resonances including the methoxy carbon resonance were identical to that obtained for **1**

Table 1. ^{13}C NMR data of flavones **1** and **2**

Carbon No.	1	2
2	163.9	163.9
3	102.4	102.3
4	182.0	182.0
5	152.3	152.2
6	131.3	131.2
7	157.1	157.0
8	94.1	94.1
9	152.7	152.6
10	104.1	104.0
1'	121.6	121.2
2'	113.4	128.2
3'	145.7	115.8
4'	149.6	160.1
5'	116.0	115.8
6'	118.9	128.2
OMe	59.9	59.8

*Present address: Delta G. Scientific P O Box 40561, Arcadia 0007.

†To whom correspondence should be addressed.

Table 2. The effects of flavones on ADP-stimulated respiration with sodium glutamate and sodium succinate as substrates.

	Sodium glutamate 10 mM			Sodium sucunate 10 mM		
Flavone	ADP:O*	ADP:O†	State 3 % Inhibition	ADP:O*	ADP:O†	State 3 % Inhibition
1 (40 μM)	2.4	1.6	45	1.5	1.3	14
2 (50 μM)	2.5	2.4	14	1.6	1.5	10

The percentage inhibition is relative to control. The control contained 25 mm^3 of ethanol and in all experiments respiratory control ratios were between 2 and 3.5.

*Adenosine diphosphate: oxygen ration in control experiment.

†Adenosine diphosphate: oxygen ration in the presence of flavone.

(±0.1 ppm) and it was thus deduced that the A and C rings were identical to **1**. The ^1H NMR spectrum was very similar to that reported previously [9].

It was observed (Table 2) that **1** and **2** inhibit ADP-stimulated respiration with both sodium glutamate and sodium succinate as substrates. It appears that the 3′-hydroxyl group is necessary for good inhibition of state 3. The fact that the ADP:O ratio in the presence of the flavanoids and in their absence are different indicated that uncoupling did occur. These two compounds were non-toxic at a dosage of 1 g/kg to mice and guinea pigs whilst ivalin, a sesquiterpene lactone which gave only 32% inhibition of state 3 under identical conditions at 1 mM level [2], was toxic to mice and guinea pigs at 0.25 g/kg subcutaneously. This unexpected result could possibly be explained by the fact that it is known that flavonoids are excreted efficiently as their glucuronides [4]. The inhibition observed with these two compounds could possibly play a role in their cytotoxicity observed against human carcinoma of the nasopharynx in cell culture [11].

EXPERIMENTAL

All the mps are uncorrected. The ^{13}C NMR spectra were recorded at 20 MHz using a Bruker WP80 spectrometer. Solns (0.25 M) in DMSO-d_6 were contained in 10 mm tubes and were run at 30°. All spectra were of 4000 Hz spectral width and free induction decays were accumulated with 16384 data locations. Pnd and sford spectra were recorded for all samples. Chemical shifts are referred to TMS.

Geigeria burkei Harv subsp. *burkei* var *zeyheri* (Harv) Merxm. was collected in the Ventersdorp district, Republic of South Africa. The plant was identified through the Courtesy of the Director, Botanical Research Institute, Pretoria.

Above-ground air-dried plant material (3 kg) was shaken twice with 96% EtOH for 24 hr. The combined extracts were dissolved in H_2O–EtOH (2:1; 3 l) and the aq. soln was extracted first with hexane and then after removal of tar, with $CHCl_3$ to give an oil (51.5 g). The oil was chromatographed in two portions on silica gel. The chromatography was controlled by TLC silica gel, 10% MeOH–$CHCl_3$) using C_6H_6, $CHCl_3$ and MeOH in different proportions. Compounds **1** (mp 277–278°) and **2** (mp 297–298°) could be isolated from MeOH–$CHCl_3$ (1:9) fractions after rechromatography on silica gel in 4% MeOH–$CHCl_3$ in yields of 2.0 g and 1.5 g (recrystallized from MeOH–$CHCl_3$) respectively.

The isolation of guinea pig liver mitochondria and the measurement of oxygen consumption with a Clark-electrode connected to a Yellow spring oxygraph have previously been described [2]. Albino guinea pigs (Wistar strain) and albino mice (indefined strain) were used and dosed with the flavonoids dissolved in propylene glycol, subcutaneously.

Acknowledgements—This work was supported in part by the Department of Agriculture. We thank Miss Sandra van Rooyen for her skilful technical assistance.

REFERENCES

1. Watt, J. M. and Breyer-Brandwijk, M. J. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa E. S.*, p. 230. Livingstone, Edinburgh.
2. Van Aswegen, C. H., Potgieter, D. J. J. and Vermeulen, N. M. J. (1982) *S. Afr. J. Sci.* **78**, 125.
3. Rimington, C., Roets, G. C. S. and Steyn, D. G. (1936) *Onderstepoort J. Vet. Sci.* **7**, 507.
4. Griffiths, L. A. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds) p. 681. Chapman & Hall, London.
5. Krishnaswamy, N. R., Seshadri, T. R. and Tahir, P. J. (1968) *Indian J. Chem.* **6**, 676.
6. Seshadri, T. R. and Sharma, P. (1973) *Indian J. Chem.* **11**, 338.
7. Herz, W. and Sumi, Y. (1964) *J. Org. Chem.* **29**, 3438.
8. Bhardwaj, D. K., Neelakantan, S. and Seshadri, T. R. (1966) *Indian J. Chem.* **4**, 173.
9. Phadke, P. S., Rao, A. V. R. and Venkataraman, K. (1967) *Indian J. Chem.* **5**, 131.
10. Markham, K. R., Chari, V. M. and Mabry, T. J. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds) p. 19. Chapman & Hall, London.
11. Kupchan, S. M., Sigel, C. W., Hemingway, R. J., Knox, J. R. and Udayamurthy, M. S. (1969) *Tetrahedron* **25**, 1603.