Bull. Chem. Soc. Ethiop. **2001**, 15(2), 137-141. ISSN 1011-3924
Printed in Ethiopia

2-HYDROXY-4-METHOXYBENZALDEHYDE: AROMATIC TASTE MODIFYING COMPOUND FROM *MONDIA WHYTEI* SKEELS

Kavaka W. Mukonyi[1] and Isaiah O. Ndiege[2*]

[1]Non-Timber Forest Products Division, Kenya Forestry Research Institute (KEFRI), P.O. Box 20412, Nairobi, Kenya
[2]Department of Chemistry, Kenyatta University, P.O. Box 43844, Nairobi, Kenya

(Received April 10, 2001; revised August 28, 2001)

ABSTRACT. 2-Hydroxy-4-methoxybenzaldehyde and 3-hydroxy-4-methoxybenzaldehyde were isolated from Mondia whytei Skeels (Asclepidiaceae). 2-Hydroxy-4-methoxybenzaldehyde was shown to have taste modifying properties. The compound was also shown to be responsible for the characteristic sweet aromatic fragrance of M. whytei root-bark. The chemical properties of 2-hydroxy-4-methoxybenzaldehyde responsible for the taste modifying character are discussed.

KEY WORDS: Asclepidiaceae, Mondia whytei Skeels, 2-Hydroxy-4-methoxybenzaldeyde, 3-Hydroxy-4-methoxybenzaldehyde, Isovanillin, Flavour, Taste-modification, Fragrance, Organoleptic test, Olfactory test

INTRODUCTION

Sucrose, a sweet natural substance whose sweetness is unmasked by any other sensation, is now identified as a major dietary component responsible for dental caries in developed countries due to its use as a bulk sweetener in foods, beverages and medicines. Much research effort has been spent on the discovery and development of high-intensity sweetening compounds with no calorific or cariogenic properties as substitutes for sucrose [1]. Synthetic sucrose substitutes such as saccharin, cyclamate and aspartame are widely used in The United States [2, 3]. Although several natural products-based commercial sucrose substitutes like phyllodulcin, stevioside, glycyrrhzin and thaumatin are used in Japan, United Kingdom, Australia and United States; questions about their safety, chemical instability, unpleasant taste characteristics, and high costs have limited their wide use and affordability [2-5].

Several indigenous communities around the world use plants in traditional sweetening and flavouring of foods, beverages and herbal medicines [6]. Their knowledge has been of immense help in the development of alternative sweeteners [4, 6, 7]. Many secondary metabolites isolated from such plants have been found to have commercial application as flavouring agents. The sweet proteinaceous principles, thaumatin I and II, have been isolated from *Thaumatococcus daniellii* Bennet (Marantaceae) [8-9] seeds traditionally used in West Africa for making over-fermented palm-wine palatable [4]. Hernadulcin, an intensely sweet bisabolone-type sesquiterpene, has also been isolated from *Lippia dulcis* Trev. (Verbenaceae) [10], used as a traditonal sweetening agent in Mexico. Dihydroquercetin-3-acetate was isolated from *Tesaria dodoneifolia* Hook and Arn. Cabrera (Compositae) and found to be responsible for the intense sweetness of the plant [1]. Stevioside, the sweet *ent*-kaurene glycoside, was isolated from *Stevia rebaudiana* Bertoni (Compositae) used traditionally in Paraguay to sweeten beverages. *S. rebaudiana* has provided seven additional sweet *ent*-kaurene glycosides [11-12]. Rubososide, another sweet *ent*-kaurene glycoside, has been isolated [13] from the leaves of *Rubus*

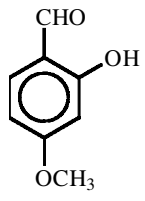
*Corresponding author. E-mail: ku-chem@clubinternetk.com

suavissimus Lee (formerly *R. chingii* Hu) (Rosaseae) a plant used for sweet caffeine-free tea in Guangxi, China. The sweet-tasting triterpene glycoside, Mongroside V, was isolated from a traditional Chinese sweet-tasting plant, *Thladiantha grosvenorii* Swingle (Curcubitaceae [1]. Another sweet-tasting triterpene, glycyrrhzin, has been isolated from the traditional sweet tasting Chinese plant, *Glycyrrhiza glabra* [14].

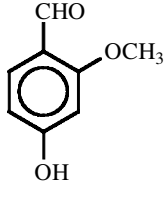
Mondia whytei (Hook) Skeels (Asclepidiaceae) is used widely in Eastern, Central and Southern Africa as a flavouring agent for porridge, tea and herbal medicines [15-16]. Besides, it is also used as an aphrodisiac by the *Luhya* of Western Kenya. Other uses include treatment of gonorrhea, malaria, infertility and enhancement of appetite, lactation in man and livestock. We hereby report the sweet fragrant principle isolated from *Mondia whytei*.

RESULTS AND DISCUSSION

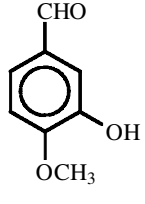
^{13}C NMR analysis of the isolated compound (**1**) showed 8 signals of which six (δ 166.8, 164.5, 135.2, 115.2, 108.4, 106.7) were due to an aromatic nucleus, one (δ 194.4) due to a carbonyl group and the remaining one (δ 55.7) due to a methoxyl group. ^{1}H NMR analysis revealed the presence of an aldehyde group (δ 9.72), a methoxyl group (δ 3.86), a chelated hydroxyl group (δ 11.48), two adjacent aromatic protons (δ 7.43, *d*, *J* = 8.7 and 6.54 Hz, *dd*, *J* = 8.7, 2.3 Hz) and one isolated aromatic proton (δ 6.43, *d*, *J* = 2.3) showing W-coupling with one of the two adjacent protons. This suggested *ortho*-, *para*- and *meta*-arrangement of the three groups (-OH, -CHO, -OCH_3) on the aromatic ring. Infra red spectroscopic analysis confirmed the presence of phenolic (3600-2500 cm^{-1}) and aldehyde (2685 cm^{-1}) groups. The presence of an aldehyde group was further confirmed by mass spectroscopy (*m/z* 151 (M^+-1), 100%). The spectral data indicated that the isolated compound could be 2-hydroxy-4-methoxybenzaldehyde (**1**), 4-hydroxy-2-methoxybenzaldehyde (**2**), 3-hydroxy-4-methoxybenzaldehyde (**3**) or 4-hydroxy-3-methoxybenzaldehyde (**4**).



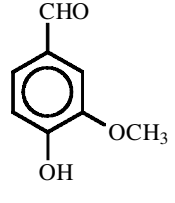
1



2



3



4

The presence of a chelated hydroxyl group evident in the ^{1}H NMR (δ 11.48) and the IR (3600-2500 cm^{-1}) strongly supported 2-hydroxy-4-methoxybenzaldehyde (**1**). A comparison of the ^{1}H NMR data of **1** with that of 4-hydroxy-3-methoxybenzaldehyde (vanillin) (**4**) revealed that the hydroxyl group in vanillin appears at δ 6.21 but at δ 11.48 in the isolated compound (**1**). Confirmation of the proposed structure was achieved by DEPT, COSY, HMQC and HMBC NMR experiments. Comparison of melting point, elemental analysis and spectral data with that of a commercial sample confirmed that the isolated compound is 2-hydroxy-4-methoxy-benzaldehyde (**1**).

Organoleptic assays confirmed that 2-hydroxy-4-methoxybenzaldehyde is responsible for the characteristic bitter-sweet taste of *Mondia whytei* roots. Olfactory assays confirmed that 2-hydroxy-4-methoxybenzaldehyde (**1**) is responsible for the characteristic sweet aromatic

fragrance of *Mondia whytei* roots. No previous taste-modifying or fragrance assays have been done on 2-hydroxy-4-methoxybenzaldehyde (**1**).

2-Hydroxy-4-methoxybenzaldehyde (**1**) was simultaneously isolated from *Mondia whytei* roots [17] and shown to be a potent tyrosinase inhibitor [18]. The compound has previously been isolated from *Periploca sepium* [19]. Cytotoxicity tests reported for 2-hydroxy-4-methoxybenzaldehyde [18] show that it is not toxic to human HTH-140 and mouse B-16 cells. However, a closely related compound, 2,4-dihydroxybenzaldehyde, inhibited tyrosinase, L-DOPA decarboxylase, induced convulsions and loss of righting reflex when injected into rabbits [20].

The only reported aromatic aldehydes with sweetening properties include vanillin (**4**), isovanillin (**3**) and cinnamaldehyde [1]. Interestingly, cinnamaldehyde does not have a hydroxyl group like the other sweet aldehydes. The presence of the •-hydroxycarbonyl arrangement in 2-hydroxy-4-methoxybenzaldehyde (**1**) (3-hydroxycarbonyl) is similar to that of hernandulcin, the sweet compound isolated from *Lippia dulcis* [10]. The only other sweet compounds or derivatives with similar arrangement include dihydroquercetin-3-acetate from *T. dodoneifolia* and the synthetic derivative, 5,7,3'-trihydroxy-4'-methoxydihydroquercetin-3-acetate [1]. Molecular mechanical calculations on 2-hydroxy-4-methoxybenzaldehyde (**1**), hernandulcin, dihydroquercetin-3-acetate and 5,7,3'-trihydroxy-4'-methoxydihydroquercetin-3-acetate have shown that the distance between the carbonyl and the proton of the hydroxyl groups is 3.689, 3.328, 1.946 and 1.946 **Å** apart, respectively, in the preferred conformations. The distance in 2-hydroxy-4-methoxybenzaldehyde (**1**) closely fits the AH, B model proposed by Shallenberger for sweet tasting compounds [21]. Similar analysis revealed that the distance between the carbonyl and the oxygen of the hydroxyl group is 2.735, 2.818, 2.735 and 2.731 **Å** in 2-hydroxy-4-methoxybenzaldehyde (**1**), hernandulcin, dihydroquercetin-3-acetate and 5,7,3'-trihydroxy-4'-methoxydihydroquercetin-3-acetate, respectively.

We also isolated trace amounts of a glycoside, 2-O-β-D-xylopyranosyl-1,6-β-D-glucopyranosyl-4-methoxybenzaldehyde, which has been previously isolated from *M. whytei* [22]. Although the isolation of the glycoside may suggest that **1** is an artifact formed during the isolation procedure, the fact that it has both the taste and the smell characteristics of the plant rules out this possibility. Both the aglycone and the glycoside may be natural products with the aglycone as a biosynthetic precursor.

Like, 2-hydroxy-4-methoxybenzaldehyde (**1**), the other compound (**3**) isolated from *Mondia whytei* also exhibited one aldehyde (δ 9.87), three aromatic (δ 7.47, *dd*; 7.46, *d* and 7.0, *d*), one hydroxyl (δ 5.75, *s*) and one methoxyl (δ 4.01, *s*) ^{1}H NMR signals suggesting that this compound could be an isomer of **1**. A comparison of ^{1}H NMR of the compound and vanillin (**4**) confirmed that it was not **4**. The melting point (112-114 ^{o}C) confirmed the isolated compound to be 3-hydroxy-4-methoxybenzaldehyde (isovanillin) (**3**). Isovanillin (**3**) is being reported from *Mondia whytei* Skeels for the first time in this paper.

EXPERIMENTAL

General procedures. Melting points (m.p.) were determined on Gallenkamp Sanyo instrument and are uncorrected. Infrared (IR) spectrometric analysis was carried out on Shimadzu FTIR machine. Elemental analysis (EA) was done on Exeter Analytical Inc. CE-440 instrument interfaced to a computer through CE-490. Mass spectroscopic (MS) data was recorded from VG Mass Lab. 12-1250 spectrometer using direct insertion probe (DIP). ^{1}H, ^{13}C, COSY, DEPT and HMQC nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ by Bruker DRX

500 MHz, Varian Gemini 300 MHz, Varian Mercury 200 MHz spectrometers. The multiplicities of the ^{13}C NMR signals were determined from DEPT experiments. Gas chromatographic analysis was done on Hewlett Packard 5890 GC using HP PONA 50 m x 0.2 mm x 0.5 μm capillary column.

Plant material. The roots of *M. whytei* Skeels were collected from Kakamega forest, Kenya, cleaned with water, debarked, the bark dried, ground and extracted.

Extraction. Dry ground *M. whytei* Skeels root-bark (350 g) was soaked in ethyl acetate: petroleum ether (1:2) for 14 hours. The mixture was filtered and the filtrate concentrated *in vacuo*. The concentrate was fractionated on silica gel to give 3.2 g (0.8%) of 2-hydroxy-4-methoxybenzaldehyde as white flakes and 10 mg of 3-hydroxy-4-methoxybenzaldehyde as a white solid. Alternatively, 2-hydroxy-4-methoxybenzaldehyde was isolated by steam distillation of *M. whytei* Skeels root-bark as a white flaky solid (crystallizing from cold water). The purity of the isolated compounds was confirmed by gas chromatographic analysis.

*2-Hydroxy-4-methoxybenzaldehyde (**1**)*. The isolated compound exhibited the following properties: found C 62.82, H 5.62, O 31.56%; m.p. 41-42 ^{o}C; MS: *m/z* 152 (M^+), 151 (100), 108, 95, 81, 80, 69, 65, 53, 51, 39; IR (KBr) ν_{max}: 3600-2500, 3108, 3028, 2986, 2884, 2851, 2685, 1662, 1578, 1518, 1500, 1477, 1441, 1391, 1350, 1336, 1300, 1240, 1184, 1167, 1140, 1020, 949, 848, 814, 799, 739, 706 cm^{-1}; ^{1}H NMR, (500 MHz, $CDCl_3$) δ 11.48 (1H, *s*, -OH), 9.72 (1H, *s*, -CHO), 7.43 (1H, *d*, *J* = 8.7 Hz, H-6), 6.54 (1H, *dd*, *J* = 8.7, 2.3 Hz, H-5), 6.43 (1H, *d*, *J* = 2.3 Hz, H-3), 3.86 (3H, *s*, OCH_3); ^{13}C NMR ($CDCl_3$) δ 194.4 (*d*, -CHO), 166.8 (*s*, C-4), 164.5 (*s*, C-2), 135.2 (*d*, C-6), 115.2 (*s*, C-1), 108.4 (*d*, C-5), 106.7 (*d*, C-3), 55.7 (*q*, -OCH_3).

*3-Hydroxy-4-methoxybenzaldehyde (isovanillin) (**3**)*. The isolated isovanillin gave the following: m.p. 112-114 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.87 (1H, *s*, -CHO), 7.47 (1H, *dd*, *J* = 8.7, 2.0 Hz, H-6), 7.46 (1H, *d*, *J* = 2.0 Hz, H-2), 7.0 (1H, *d*, *J* = 8.7 Hz, H-5), 5.75 (1H, *s*, -OH), 4.01 (3H, *s*, -OCH_3).

*4-Hydroxy-3-methoxybenzaldehyde (vanillin) (**4**)*. The commercially available vanillin gave the following spectral properties: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (1H, *s*, -CHO), 7.41 (2H, *m*, H-2, H-6), 7.03 (1H, *d*, *J* = 8.6 Hz, H-5), 6.21 (1H, *s*, -OH), 3.96 (3H, *s*, -OCH_3).

Organoleptic assay. This was done according to the procedure developed by Yoshikawa [23] but modified slightly by us. Twenty volunteers were each given 1 g of sucrose. After taking the sucrose, their mouths were thoroughly rinsed with water to get rid of the sugar taste. The same volunteers were subsequently given 20 mg of 2-hydroxy-4-methoxybenzaldehyde and 1 g of sucrose consecutively. All the 20 volunteers could not recognize the sugar taste after taking 20 mg of 2-hydroxy-4-methoxybenzaldehyde.

Olfactory assay. Twenty volunteers were blindfolded and offered identical vials containing 1 g of *M. whytei* root-bark powder and 10 mg of 2-hydroxy-4-methoxybenzaldehyde to sniff. All the twenty could not differentiate the smell of powder from that of the pure compound.

ACKNOWLEDGEMENTS

We would like to thank AAS for financial support to KWM; Prof. J. Staunton, Department of Chemistry, University of Cambridge for 500 MHz NMR and HRMS; Prof. B. Abegaz and Dr. R. Majinda, Department of Chemistry, University of Botswana for 300 MHz NMR; Mr. K.B. Wanyama, Behavioural and Chemical Ecology Department (BCED), The International Centre for Insect Physiology and Ecology (ICIPE) for GC-MS and LRMS. We also acknowledge technical assistance offered by Mr. N.W. Wairagu of Kenya Forestry Research institute (KEFRI) and Mr. E. Maina of Kenyatta University.

REFERENCES

1. Kinghorn, A.D. *J. Nat. Prod.* **1987**, 50, 1009.
2. Newburn, E. *Cariology*, 2nd ed., Williams and Wilkins: Baltimore; **1983**; pp 122-160.
3. Crosby, G.A.; Wingard Jr., R.E. in *Developments in Sweeteners*, Vol. 1, Hough, C.A.M.; Parker, J.M.; Vlitos, A.J. (Eds.), Applied Science Press: London; **1979**; pp 135-164.
4. Higginbotham, J.D. in *Developments in Sweeteners,* Vol. 1, Hough C.A.M.; Parker J.M.; Vlitos A.J. (Eds.), Applied Science Press: London; **1979**; pp 87-123.
5. Crosby, G.A.; DuBois, G.E.; Wingard Jr., R.E. in *Drug Design*, Vol. 8, Ariens, E.J. (Ed.), Academic Press: London; **1979**; pp 215-310.
6. Kinghorn, A.D.; Soejarto, D.D. in *Economic and Medicinal Plants Research*, Vol. 1, Wagner, H.; Hikino, H.; Farnsworth, N.R. (Eds.), Academic Press: London; **1985**; pp 1-52.
7. Higginbotham, J.D. in *Alternative Sweeteners*, Nabors-O'brien, L.; Geraldi, R.C. (Eds.), Mercel Dekker: New York; **1986**; pp 103-134.
8. Van der Wel, H.; Loeve, K. *Eur. J. Biochem.* **1972**, 31**,** 221.
9. De Vos, A.M.; Hatada, M.; Van der Wel, H.; Krabbendan, H.; Peerdeman, A.F., Kim, S-H. *Proc. Nat. Acad. Sci. USA* **1985**, 82, 1406.
10. Compadre, C.M.; Pezzuto, J.M.; Kinghorn, A.D.; Kamanth, S.K. *Science* **1985**, 227, 417.
11. Koyobashi, M.; Horikawa, S.; Degrandi, I.H.; Ueno, J.; Mitsuashi, H. *Phytochemistry* **1977**, 16, 1405.
12. Tanaka, O. *Saengyak Hakhoe Chi* **1980**, 11, 219; *Chem. Abstr.* **1981**, 95, 113426y
13. Tanaka, T.; Khoda, H.; Tanaka, O.; Chen, F-H.; Chou, W-H.; Leu, J-L. *Agric. Biol. Chem.* **1981**, 45, 2165.
14. Huang, K.C. *The Pharmacology of Chinese Herbs*, CRC Press: Boca Raton, Florida; **1993**; pp 275.
15. Kokwaro, J.O. *Medicinal Plants of East Africa*, East African Lit. Bureau: Nairobi; **1993**.
16. Msonthi, J.D. in *Proceedings of the 13th Plenary Meeting of the Association for the Taxonomic Study of the Flora of Tropical Africa (AETFAT)*, Seyani, J.H.; Chikuni, A.C. (Eds.), Montfort Press and Popular Publications: Limbe, Malawi; **1994**; pp 223-231.
17. Mukonyi, K.W.; Ndiege, I.O. *KIPO Patent* Appl.No. 9800260, Nairobi; **1998**.
18. Kubo, I.; Kinst-Hori, I. *Planta Med.* **1999**, 65**,** 19.
19. Sakuma, S.; Kawanishi, S.; Shoji, J.; Shibata, S. *Chem. Pharm. Bull.* **1968**, 16, 326.
20. De Ropp, R.S.; Kastl, L.; Furst, A. *Arch. Int. Pharmacodyn.* **1969**, 181, 27.
21. Shallenberger, R.S.; Acree, T.E.; Lee, Y.C. *Nature* **1969**, 221, 555.
22. Msonthi, J.D. *Bull. Chem. Soc. Ethiop.* **1991**, 5, 107.
23. Yoshikawa, K.; Kondo, Y.; Arihara, S.; Matsuura, K. *Chem. Pharm. Bull.* **1993**, 41, 1730.