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5-Hydroxylapachol: a cytotoxic agent from *Tectona grandis*

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Abstract

From the root heart wood of *Tectona grandis* were isolated a new compound, 5-hydroxylapachol (**1**) along with the known constituents lapachol, dehydro-α-lapachone, methylquinizarin and squalene. Both compound **1** and lapachol were found to be cytotoxic to *Artemia salina* (brine shrimp) with an LC_{50} of 5 ppm. The structure of compound **1** has been elucidated on the basis of its spectral data, mainly, ^{1}H and ^{13}C NMR.

Keywords: *Tectona grandis*; Verbenaceae; Naphthoquinones; 5-Hydroxylapachol; Lapachol; Dehydro-α-lapachone; Methylquinizarin; Squalene; Cytotoxicity

1. Introduction

Tectona grandis has been reported to contain 1-hydroxy-2-methylanthraquinone, tectoquinone, pachybasin, dehydrotectol, tectol, lapachol, dehydro-α-lapachone, 2-methylquinizarin, deoxylapachol, β-sitosterol, obtusifolin, squalene and betulinic acid (Thomson, 1971; Rameshwar & Seshadri, 1979; Singh, Jain, & Bhargara, 1989).

As the petrol extract of the root heartwood of *T. grandis* showed a high level of activity in our cytotoxicity tests against brine shrimps, it was investigated in order to determine the nature of the active compounds. This led to the isolation and identification of a new compound, 5-hydroxylapachol (**1**) along with the previously reported compounds lapachol, dehydro-α-lapachone, methylquinizarin and squalene. Compound **1** and lapachol were both found to be cytotoxic.

2. Results and discussion

Lapachol, dehydro-α-lapachone, methylquinizarin and squalene are known compounds and were identified by reference to reported spectral and other physical data.

Column chromatography of the concentrated petrol extract of the root heart wood, led to the isolation of a new hydroxynaphthoquinone (**1**): the UV-VIS spectrum of which showed absorption at $\lambda_{max}^{CDCl_3}$ nm (log ε): 240 (4.06), 287 (3.97) and 410 (3.52) which is similar to the general pattern observed for simple *peri*-hydroxynaphthoquinones (Thomson, 1971). The IR spectrum showed a broad hydroxy group at 3440 (cm^{-1}) and C=O absorption at 1645 (cm^{-1}) for hydroxy-1,4-naphthoquinones (Thomson, 1971).

The mass spectrum displayed the $[M]^+$ peak at m/z 258 ($C_{15}H_{14}O_4$).

In the ^{1}H NMR spectrum the chemical shifts were assigned (Table 1) by comparing the ^{1}H NMR data of **1** with those of trichione (**2**) (Kopanski, Li, Rest, & Steglich, 1982), lapachol (**3**) and 8-hydroxylapachol (**4**) (Matsumoto et al., 1985). The positions (2, 5) of the two hydroxyl groups were determined by the shift value of the chelated protons (δ 12.45, s, $CDCl_3$). Lillie, Musgrave, and Skoyles (1977) reported that the shift of the chelated hydroxy protons of 5-hydroxy-1,4-naphthoquinones (juglone type) in $CDCl_3$ is influenced by the substituents in position C-2 and C-3, but independent of the concentration of the sample. In 2,5-dihydroxynaphthoquinones the *peri*-hydroxyl proton resonates above δ 12 while in 3,5-isomers the *peri*-hydroxyl signal appears below δ 12. The prediction is

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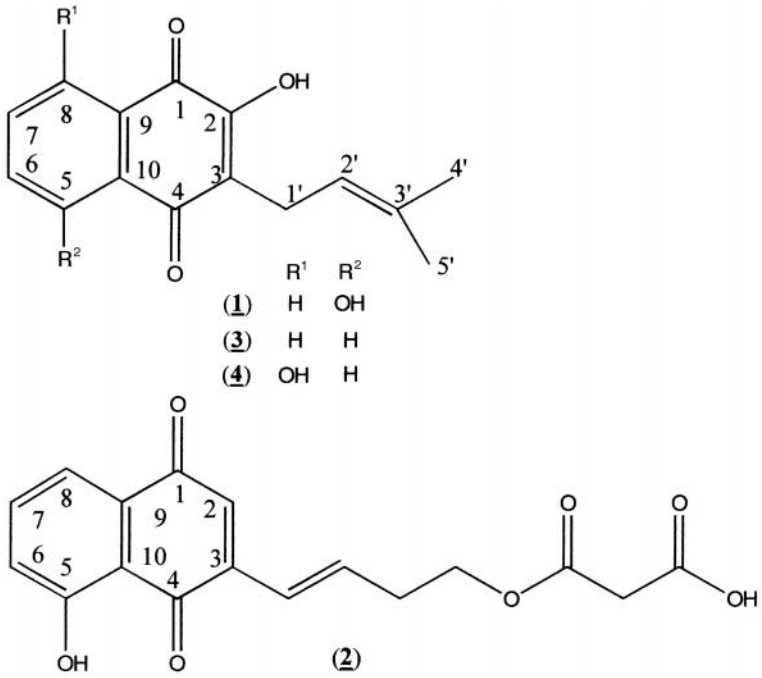

confirmed by the chemical shift values in the isomeric compound (3,5-isomer) **4** (δ 11.00) and trichione (2,5-hydroxy) (δ 12.50). The ^{13}C NMR assignments in compound **1** were made by comparison to trichione (**2**) (Kopanski et al., 1982) and lapachol (**3**) (Gafner, Wolfender, Nianga, Stoeckli-Evans, & Hostettmann 1996). C-3′ in compound **1** was assigned by comparison to the corresponding carbon in 6-methyl-5-hepten-2-one (Silverstein, Bassler, & Morrill, 1981).

2.1. Bioassay investigation

The brine shrimp lethality test was employed as an antitumour prescreen. The petrol extract of *T. grandis* root heartwood exhibited a good level of activity with an LC_{50} of 8 ppm. Compound **1** with an LC_{50} of 5 ppm was found to be as active as lapachol (LC_{50} of 5 ppm) and much superior to umbelliferone with an LC_{50} of 377 ppm. Other compounds isolated were not tested, as their activities were known and the amounts isolated were too small for screening.

Naphthoquinones in general are well known for antibacterial, antifungal and antitumoural activities and lapachol has been especially widely tested in various pharmacological studies (Goncalves de Lima, D'Albuquerque, Goncalves de Lima, & Dalia Maria, 1962; Guiraud, Steiman, Campos-Takaki, Seigle-Murandi, & Simeon de Bouchberg, 1964; Rao & Kingston, 1982; Gafner et al., 1996). Because of the highly significant antitumour activity in the Walker 256 intramuscular tumour system and of the relatively mild signs of general toxicity, lapachol was approved

Table 1
^{1}H NMR chemical shifts assignments for 5-hydroxylapachol (**1**)

H	1	2	3	4
5	–	–	8.1 *dd* (J = 6.4, 1.3)	7.60 *m*
6	7.28 *dd* (J = 6.7, 1.6)	7.30 *dd* (J = 7.3, 2.4)	7.7 *t,d* (J = 6.4, 4.0)	7.60 *m*
7	7.54 *t* (J = 7.6)	7.70 *t* (J = 7.3, 7.2)	7.7 *t,d* (J = 6.4, 4.0)	7.15 *m*
8	7.63 *dd* (J = 7.6, 1.6)	7.59 *dd* (J = 7.2, 2.4)	8.1 *dd* (J = 6.4, 1.3)	–
1′	3.28 *d* (J = 7.2)	–	3.30 *d* (J = 6.7)	3.30 *d* (J = 7.0)
2′	5.20 *t* (J = 7.2)	–	5.20 *t* (J = 6.7)	5.20 *t* (J = 7.0)
4′-CH_3^a	1.65	–	1.65	1.69
5′-CH_3^a	1.75	–	1.75	1.78
2-OH	7.39	–	7.20	–
5-OH	12.45	12.50	–	–
8-OH	–	–	–	11.00

[a]Assignments interchangeable.

by CCNSC for human clinical trials (Rao, McBride, & Oleson, 1968a). Clinically lapachol is presently used in the treatment of adenocarcinoma and squamous carcinoma (Rao, 1974; Santana et al., 1981; Almeida, Filho, Santos, & Lopes, 1990).

The activity of compound **1** is significant, as it is as good as lapachol, its structure is very similar to lapachol with an additional hydroxy group which could increase its solubility. If its general toxicity is better than lapachol then it will be a good candidate for further investigation.

3. Experimental

M.p.: uncorr.; IR: KBr; UV-VIS: $CDCl_3$; ^1H NMR: 400 MHz; ^{13}C NMR: 100 MHz; EIMS (probe) 30 eV; TLC: silica gel 60 F254 (0.22 mm thickness); prep. TLC 20×20 cm with 2 mm thickness; cc: silica gel 70–230 mesh.

Tectona grandis root heartwood was collected from the University of Dar es Salaam campus, Tanzania, in September 1991. The plant was authenticated at the Herbarium of the Botany Department, University of Dar es Salaam, where a voucher specimen is deposited.

The dried ground root heart wood (300 g) was successively extracted twice with petrol (40–60°C) (1.5 l). The petrol extract (6.2 g, 2.0%) was subjected to cc with $CHCl_3$ as eluent. This gave, in order of elution, squalene (R_f, 0.23, hexane) (59 mg, 0.02%) (Johnson & Jankowski, 1972; Pouchart, 1983) and 2-methylquinizarin (8 mg, 0.003%), m.p. 179.0–180.2°C (Thomson, 1971). The third fraction on TLC petrol–benzene (1:9) as eluent gave lapachol as fine yellow crystals (28 mg, 0.0092%), m.p. 141–142°C (Burnett & Thomson, 1968; Cannon, Joshi, & McDonald, 1975). The fourth fraction on repeated prep. TLC with petrol–benzene (1:9) yielded, on repeated recrystallization from Et_2O orange brown crystals of 5-hydroxylapachol (**1**) (R_f 0.48, $CHCl_3$) (20 mg, 0.007%), m.p. 144–145°C.

Compound **1**. UV-VIS λ_{max} nm (log ε): 240 (4.06), 287 (3.97) and 410 (3.52); IR: (KBr) ν_{max} cm^{-1}: 3417 (s) (OH), 1645 (s) (C=O), 1617 (s), 1492, 1380, 1320, 1284, 1249, 1213, 1176, 1084, 960, 900, 836, 782 and 731; ^1H NMR ($CDCl_3$): Table 1; ^{13}C NMR ($CDCl_3$): Table 2; EIMS *m/z* (rel. int): 258 $[M]^+$ (83), 244 (34), 243 (100) $[M–CH_3]^+$, 225 (11) $[M–CH_3–H_2O]^+$, 216 (6), 215 (23) $[M–CH_3–CO]^+$, 197 (8) [215-H_2O, 225-CO], 195 (8), 187 (8), 175 (8), 121 (15), 115 (10), 92 (8), 77 (10), 65 (8), 53 (8), 41 (13), 39 (13). Found: C, 46.49; H, 5.44, $C_{15}H_{14}O_4$ requires: C, 46.51; H, 5.42.

The final fraction on repeated chromatography deposited orange needles of dehydro-α-lapachone (15 mg, 0.005%), m.p. 142–144°C (Burnett & Thomson, 1968; Cannon et al., 1975).

Table 2
^{13}C NMR chemical shifts assignments for 5-hydroxylapachol (**1**)

C	1	2	3
1	180.9	180.2	–
2	153.3	155.5	–
3	123.1	117.1	–
4	190.7	190.4	–
5	161.4	160.6	–
6	126.3	124.9	–
7	135.0	135.4	–
8	119.4	118.4	–
9	129.4	130.3	–
10	114.5	113.8	–
1′	25.8	–	5.7
2′	119.2	–	19.6
3′	134.2[a]	–	–
4′[b]	16.9	–	17.9
5′[b]	22.0	–	2.6

[a]Assigned by comparison to the corresponding carbon in 6-methyl-5-hepten-2-one Guiraud, Steiman, Campos-Takaki, Seigle-Murandi and Simeon de Bouchberg (1964), **2** and **3**.
[b]Assignments interchangable.

3.1. Assay of cytotoxicity

The lethality towards *Artemia salina* (brine shrimp) using the procedure of Meyer, Ferrigni, Putnam, Jacobsen, and Nichols (1982) was employed. Lapachol, an active antitumour (Rao, McBride, & Oleson, 1968b) agent, was used as a standard along with umbelliferone (Doganca, Gurkan, Hirlak, Tuzun, & Tuzlaci, 1997).

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References

Almeida, E. R. D., Filho, A. A. D. S., Santos, E. R. D., & Lopes, C. A. C. (1990). *J. Ethnopharmacol., 29,* 239.
Burnett, A. R. and Thomson, R. H., *J. Chem. Soc. C*, 1968, 850–853.
Cannon, J. K., Joshi, K. R. and McDonald, I. A., *Tetrahedron Lett.*, 1975, 2795-5.
Doganca, S., Gurkan, E., Hirlak, F., Tuzun, O. T., & Tuzlaci, E. (1997). *Fitoterapia, LXVIII,* 80.
Gafner, S., Wolfender, J. L., Nianga, M., Stoeckli-Evans, H., & Hostettmann, K. (1996). *Phytochemistry, 42,* 1315.
Goncalves de Lima, O., D'Albuquerque, I. L., Goncalves de Lima, C., & Dalia Maria, M. H. (1962). *Rev. Inst. Antibiot. (Recife), 4,* 3.

Guiraud, P., Steiman, R., Campos-Takaki, G. M., Seigle-Murandi, F., & Simeon de Bouchberg, M. (1964). *Planta Med., 60,* 373.

Johnson, L. F., & Jankowski, W. C. (1972). *Carbon-13 NMR spectra.* New York: John Wiley and Sons.

Kopanski, L., Li, G. R., Best, H., & Steglich, W. (1982). *Liebigs Ann. Chem*., 1722.

Lillie, T. J., Musgrave, O. C., & Skoyles, D. (1977). *J.C.S. Perkin Trans. I*, 355.

Matsumoto, T., Ichihara, A., Yanagiya, M., Yuzawa, T., Sannai, A., Oikawa, H., Sakamura, S., & Eugster, C. H. (1985). *Helv. Chim. Acta, 68,* 2324.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., & Nichols, D. E. (1982). *Planta Med., 45,* 31.

Pouchart, C. (1983). *The Aldrich library of NMR spectra* (2nd ed.) (p. 36c). Aldrich.

Rameshwar, J., & Seshadri, T. R. (1979). *J. Indian Chem. Soc., 56,* 940–941.

Rao, K. V. (1974). *Cancer Chemother. Rep., 4 (Part 2),* 11.

Rao, M. M., & Kingston, D. G. I. (1982). *J. Nat. Prod., 45,* 600.

Rao, K. V., McBride, T. J., & Oleson, J. J. (1968a). *Cancer Res., 28,* 1952.

Rao, K. V., McBride, T. J., & Oleson, J. J. (1968b). *Cancer Res., 28,* 1952.

Santana, C. F., Lins, L. J. P., Asfora, J. J., Melo, A. M., Lima, G., & D'Albuquerque, I. L. (1980–1981). *Rev. Inst. Antibiot., 20,* 61.

Silverstein, R. M., Bassler, G. C., & Morrill, T. C. (1981). *Spectroscopic identification of organic compounds* (4th ed.). New York, Chichester, Brisbane, Toronto: John Wiley and Sons.

Singh, P., Jain, S., & Bhargara, S. (1989). *Phytochemistry, 28,* 1258–1289.

Thomson, R. H. (1971). *Naturally occurring quinones* (2nd ed.) London and New York: Academic Press.