extracted with methanol The residue obtained by evaporation of the solvent was partitioned between ethyl acetate and water The organic extract was dried and evaporated The residue was chromatographed on silica gel and the sterol fraction was fractionated further by HPLC Each subfraction was analysed by GC and GC/MS leading to the identification of the sterols of the mixture (see Table 1) by comparison with known standards As it has been normally found in red algae, cholesterol was by far the most abundant constituent of the mixture, which also contained C_{28} and C_{29} saturated and unsaturated components It is interesting to note that desmosterol was present, in small amount, in the free state but we were unable to detect any side chain hydroxylated sterol

EXPERIMENTAL

General HPLC was carried out on a Whatman Partisil M9 10/50 ODS-2 column using an RI detector (mobile phase MeOH) GC was on a fused silica capillary column (12 m × 0 02 mm) coated with methyl silicone fluid (Hewlett–Packard) Computerized GC/MS was on a Varian-Mat CH7-A instrument at 70 eV

Plant material Gigartina skottsbergii was collected at Puerto Madryn, Chubut, Argentina in winter at 8 m depth Voucher specimens have been deposited at the Centro Nacional Patagónico, Puerto Madryn

Extraction and analysis Fresh algae (3 4 kg) were freeze-dried and the residue (1 2 kg) was extracted with MeOH (2 × 2 l) yielding an extract which was partitioned between EtOAc and H_2O The syrup obtained by evapn of the organic solvent (4 45 g) was chromatographed twice on silica gel columns eluted with toluene and toluene–CH_2Cl_2 (1 1) to separate the free sterol fraction This fraction (99 mg) was purified by HPLC yielding four subfractions that were analysed by GC and GC/MS techniques Identification of each sterol was made by comparison with the MS data of authentic samples (see Table 1)

Acknowledgements—We thank Dr A Borasso (Centro Nacional Patagónico) for collection and classification of the algae and Prof C Djerassi (Stanford University) for authentic samples We are also indebted to the Organization of the American States for financial support

REFERENCES

1 Goodwin, T W (1974) in *Algal Physiology and Biochemistry* (Stewart, W D, ed), Botanical Monographs, Vol 10 Blackwell Scientific Publications, London
2 Goad, L J (1978) in *Marine Natural Products Chemical and Biological Perspectives* (Scheuer, P J, ed), Vol II, Chap 2 Academic Press, New York
3 Amico, V, Chillemi, R, Sciuto, S, Tringali, C, Cormaci, M and Furnari, G (1982) *Naturalista Sicil* S IV, VI (Suppl), 1, pp 95–106
4 Combaut, G, Codomier, L, Teste, J and Pedersen, M (1981) *Phytochemistry* **20**, 1748
5 Francisco, C, Combaut, G, Teste, J, Tarchini, C and Djerassi, C (1979) *Steroids* **34**, 163
6 Kabore, S A, Combaut, G, Vidal, J P, Codomier, L, Passet, J, Girard, J P and Rossi, J C (1983) *Phytochemistry* **22**, 1239

Phytochemistry, Vol 23, No 11, pp 2689–2691, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

ANTHRAQUINONES FROM *CASSIA SOPHERA* ROOT BARK

A DASS, T JOSHI and S SHUKLA*

Department of Chemistry, University of Allahabad, Allahabad, India, *Department of Pharmacology, M L N Medical College, Allahabad, India

(*Revised received* 12 *April* 1984)

Key Word Index—*Cassia sophera*, Leguminosae, 1,8-dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone, 1,3-dihydroxy-5,7,8-trimethoxy-2-methylanthraquinone

Abstract—Two new anthraquinones have been isolated from the root bark of *Cassia sophera* and characterized as 1,8-dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone and 1,3-dihydroxy-5,7,8-trimethoxy-2-methylanthraquinone

Cassia sophera (Leguminosae) is well known for its medicinal value [1], roots, flowers and heartwood have been studied chemically [2–6]

From the benzene fraction of the acetone extract of the root bark of *Cassia sophera* two new anthraquinones have now been isolated and characterized by spectral and chemical studies Both compounds (**1** and **2**) responded to characteristic colour tests for anthraquinones Their λ_{max} and IR spectra supported the above conclusion Strong peaks at 1460 cm^{-1} in the IR spectrum of **1** and at 1450 cm^{-1} in that of **2**, a signal at δ2 35 in the ^1H NMR spectrum of **1** and 2 38 in that of **2** and identification of 2-

methylanthracene on zinc dust distillation indicated a methyl group at the β-position in both compounds Presence of two hydroxyl groups (peak at 3400 cm^{-1} in IR and formation of a diacetate) was also observed in both

In **1**, molecular formula $C_{19}H_{16}O_6$, mp 261° (dec), the presence of two methoxyl groups was also observed [7] A strong peak at 750 cm^{-1} in the IR spectrum for C=C suggested the presence of an unsaturated side chain in the molecule Unsaturation estimation [8] and signals at δ4 6 and 4 7 (*s*, 3H of $-CH=CH_2$) in the ^{1}H NMR spectrum confirmed the presence of a vinyl group The formation of a copper complex showed the presence of chelated hydroxyl, i e a hydroxyl α to the carbonyl [9] Its λ_{max} at 430 nm indicated the presence of at least two hydroxyl groups in the α-position Two strong peaks in the IR at 1665 and 1620 cm^{-1} and positive response to the colour tests with alkali and formamide [10] and alkaline zirconium nitrate [11] confirmed a 1,8-dihydroxy system To ascertain the positions of the methoxyl groups the compound was demethylated with hydriodic acid and red phosphorus The demethylated product contained four hydroxyl groups, since it gave a tetra-acetate It gave a pink colour with methanolic magnesium acetate suggesting a 1,3-dihydroxy system [12], so one of the methoxyls must be at position 3 Negative tests for a 1,4- or a 1,5-dihydroxy system [13] along with a signal at δ7 3 (for protons at positions 4 and 5) suggested that positions 4 and 5 were unsubstituted, so the second methoxyl must be at position 6 or 7 The demethylated product did not give colour with ammonium molybdate and acetic acid [14] showing the absence of a catechol unit This eliminated the possibility of hydroxyl being at position 7 in the demethylated compound, so that the vinyl group must be at 7 and the hydroxyl (and hence the second methoxyl) must be at position 6 This was further confirmed by permanganate oxidation of the demethylated product Neutral permanganate oxidation of the compound for 15 hr gave only one product identified as 3,5-dihydroxytrimellitic acid by direct comparison with a synthetic sample (mmp 104°, co-TLC, λ_{max}^{EtOH} 220 nm, and superimposable IR) Absence of a free catechol unit in the oxidation product, confirms the position of the second methoxyl as 6 Hence **1** can be characterized as 1,8-dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone This is the first report of the compound in nature

Compound **2**, molecular formula $C_{18}H_{16}O_7$, mp 305° (d) revealed the presence of three methoxyl groups by quantitative estimation [7], supported by the signal at δ3 9 in its ^{1}H NMR spectrum [15], peaks at 2840 [16] and 1110 cm^{-1} in the IR [17, 18] The formation of a copper complex indicated the presence of a α-hydroxyl group This is supported by its λ_{max} at 412 nm Positive response to the methanolic magnesium acetate test indicated the presence of two hydroxyls at the 1,3-positions The presence of β-hydroxyl group was further supported by its solubility in sodium carbonate

Chromic acid oxidation of **2** yielded 3,4,6-trimethoxyphthalic acid which was identified by direct comparison with an authentic sample (mmp, co-TLC and superimposable IR) Identification of 3,4,6-trimethoxyphthalic acid indicates that all the three methoxyl groups are present on one ring and two definitely at positions 5 and 8 (corresponding to 3 and 6 positions of 3,4,6-trimethoxyphthalic acid) The third methoxyl may be at position 6 or 7 The position of the third methoxyl was determined by the IR spectrum of the demethylated product which revealed only one very strong absorption peak at 1580 cm^{-1} confirming the presence of 1,7-dihydroxy system [19] So the third methoxyl is present at 7 Hence **2** is 1,3-dihydroxy-5,7,8-trimethoxy-2-methylanthraquinone An anthraquinone of this substitution pattern has not been reported before in literature

EXPERIMENTAL

The root bark of *Cassia sophera* was extracted with hexane and Me_2CO successively The concd Me_2CO extract was fractionated into petrol, C_6H_6, Et_2O, EtOAc and MeOH soluble portions The C_6H_6 fraction yielded **1** and **2**, which were separated by prep TLC

Compound **1** Yellow crystals, mp 261° (dec), molecular formula $C_{19}H_{16}O_6$, Found C, 67 04%, H, 4 7%, OMe, 18 07%, requires C, 67 05%, H, 4 70%, OMe, 18 23% UV λ_{max}^{EtOH} nm 270 (sh), 288, 322 (sh) and 430 IR ν_{max}^{KBr} cm^{-1} 3350, 3010, 2855, 1675, 1620, 1460, 1380, 1310, 1265, 1110, 1036, 995 and 750 ^{1}H NMR (90 MHz, $CDCl_3$) δ2 35 (*s*, 3H, Me), 3 8 (*s*, 6H, 2 × OMe at C-3 and C-6), 4 6 and 4 7 (*s*, *s*, 1H, 2H, $-CH=CH_2$) and 7 2 (2H, H-4 and H-5) Diacetate (pyridine, Ac_2O, 24 hr at room temp) mp 245° (found Ac, 20 15%, $C_{19}H_{14}O_6$ $(Ac)_2$ requires 20 28%) Unsaturation estimated 8 7%, $C_{17}H_{13}O_6$ $(CH=CH_2)$ requires 8 65% Demethylated product crystallized from EtOAc–light petrol, mp 269°, formed tetra-acetate (Ac 35 6%, $C_{17}H_8O_6$ $(Ac)_4$ requires 35 8%)

Neutral $KMnO_4$ *oxidation* Compound **1** (50 mg) in Me_2CO (50 ml) was refluxed with excess of $KMnO_4$ for 15 hr Excess of Me_2CO distilled off MnO_2 was dissolved by passing SO_2 gas and the clear soln extracted with Et_2O The Et_2O extract was shaken with $NaHCO_3$ and acidified with HCl It was again extracted with Et_2O The residue on crystallization from Et_2O had mp 104°, λ_{max}^{EtOH} 220 nm, identified as 3,5-dihydroxytrimellitic acid by direct comparison with an authentic sample (mmp, co-TLC and superimposable IR)

Synthesis of 3,5-*dihydroxytrimellitic acid* It was prepared by heating one mole of 2,6-dihydroxytoluene with three moles of chloroacetyl chloride in presence of dry $AlCl_3$ for 15 hr The reaction product was then oxidized with acidic $KMnO_4$ to give 3,5-dihydroxytrimellitic acid, mp 104° Found C 44 85%, H, 2 4%, Calc for $C_9H_6O_8$, C, 44 6%, H, 2 4% IR ν_{max}^{KBr} cm^{-1} 3430, 3010, 2980, 1710, 1640, 1610, 1520, 1400, 1200, 1060 and 910

Compound **2** Yellow crystals, mp 305° (dec), molecular formula $C_{18}H_{16}O_7$ Found C, 4 65%, H, 4 55%, OMe, 26 9%, requires C, 62 79%, H, 4 65%, OMe, 27 03%, UV λ_{max}^{EtOH} nm 275, 290, 328 (sh) and 412 IR ν_{max}^{KBr} cm^{-1} 3350, 3000, 2840, 1690, 1450, 1375, 1315, 1110, 1032 ^{1}H NMR (90 MHz, $CDCl_3$) δ2 38 (*s*, 3H, Me), 3 9 (*s*, 9H, 3 × OMe at 5, 7 and 8), 7 2 (*s*, 1H, H-6), 7 7 (*s*, 1H, H-4) Acetate (pyridine–Ac_2O, 24 hr at room temp) mp 278° (Found Ac 19 99%, $C_{18}H_{14}O_7$ $(Ac)_2$ requires 20 09%)

Chromic acid oxidation Compound **2** (20 mg) in Ac_2O (3 ml) and HOAc (1 ml) were refluxed for 0 5 hr CrO_3 (300 mg) in HOAc (3 ml) was added and refluxed for 2 hr After work up, the product was extracted into Et_2O and then crystallized It was identified as 3,4,6-trimethoxyphthalic acid by direct comparison with an authentic sample (mmp, 185°, co-TLC and superimposable IR)

REFERENCES

1 Kirtikar, K R and Basu, B D (1940) *Indian Medicinal Plants*, Vol III, p 863 Leader Press, Allahabad, India
2 Tiwari, R D and Misra, G (1975) *Planta Med* **28**, 182
3 Misra, G (1972) D Phil Thesis, Department of Chemistry,

University of Allahabad, Allahabad, India
4 Kartha, A R S (1963) *Indian J Chem.* **5**, 67
5 Malhotra, S and Misra, K (1982) *Phytochemistry* **21**, 197
6 Malhotra, S and Misra, K (1982) *Planta Med* **46**, 247
7 Ziesel, S (1885) *Monash* **8**, 989
8 Belcher, R and Godbert, A I (1960) *Semimicro Methods of Organic Analysis*, p 133 Green & Co, New York
9 Somogyi, (1952) *J Biol Chem* **19**, 15
10 Lemli, J, Dequeker, R and Cuvelee (1969) *J Pharm Weekblad* **99**, 351
11 Feigl, F and Anger, V (1966) *Spot Tests in Organic Analysis* p 347 Elsevier, Basel
12 Shibata, S, Takido, M and Tanaka, O (1950) *J Am Chem Soc* **72**, 2789
13 Raistrick, H and Ziffer, J (1951) *Biochem J* **49**, 563
14 Geissman, T A (1962) *The Chemistry of Flavonoid Compounds* Pergamon Press, New York
15 Sargent, M V, David, Smith, O N, Elix, J A and Roffey, P (1969) *J Chem Soc (C)* 2766
16 Hill, R D and Meakins, G D (1958) *J Chem Soc* 760
17 Bell, J V, Heisler, J, Tannerbaum, H and Goldensor, J (1953) *Analyt Chem.* **25**, 1720
18 Ory, H A (1960) *Analyt Chem* **32**, 509
19 Bick, I R C and Rhee, C (1966) *Biochem J* **98**, 112

Phytochemistry, Vol 23, No 11, pp 2691–2692, 1984
Printed in Great Britain

0031-9422/84 $3 00 + 0 00

2,4,2′-TRIHYDROXY-4′-METHOXYBENZIL FROM *ZOLLERNIA PARAENSIS**

F FERRARI, R ALVES DE LIMA† and G B MARINI BETTOLO

Centro Chimica del Recettori e delle Molecole Biologicamente Attive del C N R, Università Cattolica del S Cuore Largo F Vito, 1 00168 Roma, Italy, †Depto de Quimica, U F A L, 57 000 Maceio, Brazil

(*Revised received* 23 *March* 1984)

Key Word Index—*Zollernia paraensis*, Leguminosae, 2,4,2′-trihydroxy-4′-methoxybenzil

Abstract—A new phenolic compound, isolated from the wood of *Zollernia paraensis*, has been shown to be 2,4,2′-trihydroxy-4′-methoxybenzil by spectral data and synthesis

INTRODUCTION

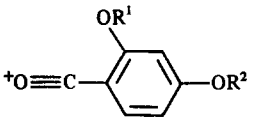
In previous papers [1, 2], we reported the isolation of ten flavonoids from a benzene extract of *Zollernia paraensis* Hub Further studies of the more polar fraction led to the isolation of a new compound, 2,4,2′-trihydroxy-4′-methoxybenzil (**1**) Benzils have not been isolated so far from natural sources, but have been obtained only as degradation products of lignins [3, 4]

RESULTS AND DISCUSSION

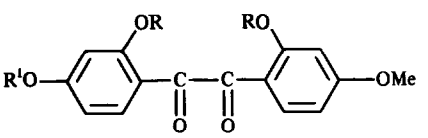
The ^{1}H NMR spectrum of **1**, $C_{15}H_{12}O_6$ (M^+ at *m/z* 288), showed signals for a methoxy group (δ3 90), six aromatic protons (δ6 50–7 50) of two 1,2,4-trisubstituted rings and one or two chelated hydroxyl groups (*br*, δ11 0–11 50) Methylation of **1** with methyl iodide gave the trimethyl ether **2**, $C_{18}H_{18}O_6$ (M^+ at *m/z* 330), the ^{1}H NMR spectrum of which showed a new methoxyl group coincident with the methoxyl group of **1** (δ3 85), while the other two methoxy groups resonated upfield (δ3 55)

The fragmentation pattern of **1** in the mass spectrum showed two major ions at *m/z* 151 (**a**, 100%) and 137 (**b**, 90%), which were both shifted to *m/z* 165 (**c**, 100%) in the mass spectrum of the methyl derivative **2** Consideration of the ^{1}H NMR spectra and the typical MS fragmentation of benzils [5] suggested for the three ions the structures **a**–**c**, and consequently structure **1** for the natural product

A benzil structure was confirmed by oxidative cleavage of **2** with alkaline hydrogen peroxide to give 2,4-dimethoxybenzoic acid Direct synthesis of compound **1** by oxidation of the corresponding benzylated chalcone by TTN in perchloric acid [6] was unsuccessful, as was the attempted synthesis of the trimethyl ether of **1** via benzoin [7] Conversely compound **2** was synthesized by reaction



	R¹	R²
a	H	Me
b	H	H
c	Me	Me



	R	R¹
1	H	H
2	Me	Me
3	H	Me
4	Ac	Ac

*Part 3 of the series "Flavonoids and Isoflavonoids from *Zollernia paraensis*" For part 2 see ref [2] A preliminary communication of this work was presented at the 2nd International Conference on Chemistry and Biotechnology of Biologically Active Natural Products, Budapest, 1983