TABLE 1. R_fS AND SPECTRAL PROPERTIES OF ANTHOCYANINS OF *Asarum asaroides*

Pigment	Absorption spectra* λ_{max} (nm)			$\frac{E_{acyl\ peak}}{E_{vis.\ max}}$	R_fs† (×100) in BuH	Forestal	BAW
Peonidin:							
3-*p*-Coumaroylgentiobioside	527	315	284	0·67	63	69	26
3-Caffeoylgentiobioside	527	333	283	0·54	45	59	13
Cyanidin:							
3-*p*-Coumaroylgentiobioside	529	316	284	0·66	63	61	23
3-Caffeoylgentiobioside	529	333	283	0 53	45	51	11
Deacylated pigments:							
Peonidin 3-gentiobioside	522		277		32	74	18
Cyanidin 3-gentiobioside	524		280		27	63	13

* In MeOH containing 0·01% HCl.

† Determined on Tôyô-roshi No. 51 paper. BAW = BuOH–HOAc–H_2O (6:1:2), see Experimental for other solvents.

EXPERIMENTAL

The anthocyanin extract of flowers was separated into two bands by paper chromatography in BuOH–HCl–H_2O (7.2:5) (BuH). Each band was further separated into two components by chromatography in HOAc–HCl–H_2O (30:3:10) (Forestal). The latter procedure was repeated twice in order to purify each pigment completely. The four anthocyanins separated were examined by standard procedures as described earlier.[2]

Acknowledgements—The author's sincere thanks are due to Professor M. Shibata (Toyama Women's Junior College) for providing the authentic specimens of peonidin and peonin.

[2] N. ISHIKURA, *Bot. Mag. Tokyo* **83,** 179 (1970).

Phytochemistry, 1971, Vol. 10, pp 3298 to 3302. Pergamon Press Printed in England.

BIGNONIACEAE

5,6,7-TRIMETHOXYFLAVONE AND 5,6,7,8-TETRAMETHOXYFLAVONE FROM *ZEYHERA TUBERCULOSA*

JAMES P. KUTNEY and HARALD W. HANSSEN

Department of Chemistry, University of British Columbia, Vancouver 8, Canada

(*Received* 14 *April* 1971, *in revised form* 4 *June* 1971)

Abstract—The major constituents from an ethanolic extract of the leaves of *Zeyhera tuberculosa* Bur. ex. Verlot were shown to be 5,6,7-trimethoxyflavone (III) and 5,6,7,8-tetramethoxyflavone (IV).

INTRODUCTION

DUE TO the relative inaccessibility of fresh plant material, phytochemical studies of tropical species of the Bignoniaceae have been limited.[1,2] Some species of Bignoniaceae have been

[1] (a) F. EARL, *J. Am. Oil Chem. Soc.* **37,** 440 (1960); (b) M. J. CHISHOLM and C. Y. HOPKINS, *Can. J Chem.* **43,** 2566 (1965).

[2] For a recent review and summary of references see, J B. HARBORNE, *Phytochem.* **6,** 1643 (1967).

investigated for certain therapeutical applications.[3] A recent report[4] that the extracts of *Zeyhera tuberculosa*, an arborescent species native to Brazil, appear to have anti-tumour properties stimulated our interest in investigating the chemical constituents of this species.

RESULTS AND DISCUSSION

The benzene-neutral portion of ethanolic leaf extract of the above plant was shown to contain four components A–D in order of increasing polarity on silica gel plates. Compounds A and B were very minor components which were difficult to purify and attention was therefore directed at C and D.

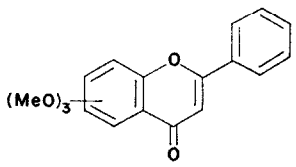
Compound D, m.p. 172–172·5°, from elemental analysis and high resolution mass spectrometric measurement, had the molecular formula $C_{18}H_{16}O_5$. The UV spectrum showed maxima at 272 and 304 nm and showed no significant change on addition of alkali thereby excluding the presence of enolic or phenolic hydroxyl groups. The main feature in the IR spectrum was a strong carbonyl absorption at 1625 cm^{-1}, rather characteristic of a γ-pyrone carbonyl group in flavones. The NMR spectrum of D revealed the presence of signals due to three aromatic methoxyl groups (τ6·09, 6H, singlet, and 6·15, 3H, singlet) while signals at low field (τ 2·22, 2H, multiplet, and τ 2·59, 3H, multiplet) were in excellent agreement with those normally assigned to flavone ring B protons. Finally, absorptions at τ 3·28 (1H, singlet) and τ 3·42 (1H, singlet) provided further suggestion of a flavone system.[5]

The mass spectrum of compound D again revealed a fragmentation pattern typical of flavones.[6–8] The most prominent cleavage was the loss of a methyl radical from the parent ion at *m/e* 312 to give rise to the base peak at *m/e* 297. The latter then showed a loss of 28 mass units (probably CO) to give the next prominent peak at *m/e* 269. Subsequent homolysis of the latter fragment proceeded via several pathways. Thus cleavage to the ions at *m/e* 102 (phenyl acetylene) and *m/e* 167 was an expected fragmentation of a flavone skeleton. Similarly loss of a methyl radical from the ion at *m/e* 269 provided *m/e* 254 and the latter could then undergo cleavage to *m/e* 102 and the ion at *m/e* 152. All the above data suggested that compound D had partial structure (I).

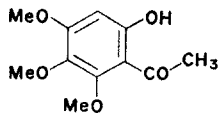
To provide chemical evidence in support of (I), compound D was submitted to alkaline hydrolysis, a reaction which normally converts the flavone ring to *o*-hydroxyacetophenone derivatives.[9] The major reaction product exhibited spectral data (see Experimental) in complete accord with that expected for 2,3,4-trimethoxy-6-hydroxyacetophenone (II). Conclusive proof for the structure of the hydrolysis product came forth from its synthesis (see Experimental). The synthetic material was identical (TLC, IR and NMR spectra) with the compound obtained from the alkaline hydrolysis of compound D. This data therefore established D as 5,6,7-trimethoxyflavone (III), the first recorded natural occurrence of the trimethyl ether of baicalein (5,6,7-trihydroxyflavone). The synthesis of 5,6,7-trimethoxy-

[3] J. KERHARO and A. BOUQUET, *Bull. Soc. Bot. Fr.* **94**, 251 (1947).
[4] Private communication from Dr. B. GILBERT, Rio de Janeiro, Brazil. We are grateful to Dr. Gilbert for informing us of his results.
[5] For a recent compilation of spectral data see, T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York (1970).
[6] R. I. REED and J. M. WILSON, *J. Chem. Soc.* 5949 (1963).
[7] C. S. BARNES and J. L. OCCOLOWITZ, *Austral. J. Chem.* **17**, 975 (1964).
[8] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 262, Holden-Day, San Francisco (1964).
[9] F. M. DEAN, *Naturally Occurring Oxygen Ring Compounds*, p. 291, Butterworths, London (1963).

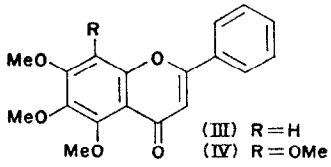
flavone has been reported from several laboratories[10,11] but samples for comparison were unavailable.



(I)



(II)



(III) R=H
(IV) R=OMe

Compound C, $C_{19}H_{18}O_6$, the major component from the benzene-soluble neutral fraction, revealed spectral data which suggested a close similarity with D. Thus its UV spectrum was identical with that of D, while the IR spectrum indicated a strong absorption at 1655 cm^{-1}. The NMR spectrum established the presence of *four* aromatic methoxyl groups (τ 5·90, 3H; 5·96, 3H; 6·03, 6H) while aromatic proton absorptions (τ 2·1, 2H; 2·5, 3H) showed a pattern virtually identical to that of D thereby suggesting that C was 5,6,7,8-tetramethoxyflavone (IV).

The mass spectrum of C established the correctness of this assignment. The fragmentation pathway was identical with that of D *except* that all the relevant fragments appear at values which are 30 mass units higher. These data leave no doubt that the additional methoxyl group is in ring A and thereby the structure of C is confirmed as (IV). This is the first reported isolation of this compound from a natural source.

EXPERIMENTAL

TLC was carried out using either Merck silica gel or Woelm neutral alumina; electronic phosphor (about 2% by weight) was added as fluorescent indicator. The chromatoplates were developed in $CHCl_3$ or $CHCl_3$–2% MeOH. Column chromatography was performed on Woelm silica gel, deactivated by the addition of 10% H_2O.UV spectra were recorded in MeOH and IR spectra as KBr pellets or in $CHCl_3$. The positions of absorption maxima are quoted in wave numbers (cm^{-1}).

NMR spectra were measured in $CDCl_3$. The positions of the signals are given in the Tiers τ scale with tetramethylsilane as the internal standard at τ 10·00. For multiplet signals the given values represent the center of the signal. Mass spectra were measured on an AEI MS-902 mass spectrometer Fragmentation data is given in mass to charge ratios (*m/e*) followed by percent relative abundance.

M.ps were determined on a Kofler block and are uncorrected. Elemental analyses were performed by Mr. P. Borda of the Microanalytical Laboratory, University of British Columbia, Vancouver.

Initial separation of the ethanolic extract of Z. tuberculosa. Leaves of *Z. tuberculosa* were picked in the vicinity of Rio de Janeiro, Brazil. After crushing, they were extracted with hot ethanol. Removal of most of the solvent left a dark green residue

This extract (25 g) was dissolved in warm water (500 ml) and extracted three times with benzene (1 l.). Removal of the solvent *in vacuo* yielded a dark green gum (18·6 g). The aqueous layer was then extracted three times with $CHCl_3$ (1 l.) to give a brown gum (2·7 g). Finally, the aqueous layer was extracted with Et_2O (1 l.) to give another brown gum (0·4 g). Finally evaporation of the remaining aqueous layer yielded a brown, semi-crystalline residue (0·3 g) with which no further work was done.

Each of these fractions was dissolved in $CHCl_3$ and treated in the following manner: First the extract was washed several times with aqueous 10% NH_4OH. This aqueous layer was then acidified with 10% HOAc and extracted with $CHCl_3$. Evaporation of solvent yielded a residue containing the acidic components.

The initial $CHCl_3$ extract was then washed several times with 10% HOAc. The aqueous layer was again extracted with $CHCl_3$, and removal of solvent yielded a residue containing the basic material. Finally evaporation of the original organic layer gave a neutral residue.

TLC of the benzene-soluble neutral fraction. Chromatography of a sample of this fraction on silica gel with $CHCl_3$–1% MeOH showed that this fraction consisted mainly of two closely running compounds (dark

[10] V. D. NAGESWARA SASTRI and T. R. SESHADRI, *Proc. Ind. Acad. Sci.* **23A**, 253, 262 (1946).
[11] A. OLIVERIO, G. B. MARINI-BETTOLO and G. BARGELLINI, *Gazz. Chim. Ital.* **78**, 363 (1948); *ibid.* **80**, 789 (1950).

blue spot in UV, R_f 0·35, and yellow spot in UV, R_f 0·30), and also considerable front running material. Subsequent chromatography of this least polar component on silica gel using benzene showed this to consist of two compounds. Thus, these four compounds were labelled in order of increasing polarity as A, B, C and D.

Column chromatography of the benzene-soluble neutral fraction. A sample of the extract (5·0 g) was dissolved in a minimum amount of benzene and chromatographed on silica gel (500 g, 10% H_2O). Elution with benzene (700 ml) yielded a slightly yellow oil (0·34 g) which was named Compound A. Further elution with benzene (750 ml) gave an orange and partly-crystalline gum (0·4 g), called Compound B. The column was then eluted with ether (1 l.) to give a green gum (3·2 g) consisting mainly of Compounds C and D.

The above gum (3·2 g) obtained from the initial chromatography on silica gel was dissolved in benzene and chromatographed on alumina (150 g). Elution with benzene (1 l.), benzene–ether (9:1), and benzene–ether (8:2, 1 l.) provided C (2·0 g) as a crystalline compound. Further elution with this latter solvent gave another white crystalline fraction (0·4 g) which was named D.

Properties of compound C. This material (2·0 g) was recrystallized from Et_2O as long colourless needle-like crystals (1·9 g), m.p. 116·0–116·5°, λ_{max}: 271, 304 nm (no significant change in 0·002 M $NaOCH_3$ or on addition of $AlCl_3$ (log ϵ 3 23); ν(KRr): 2995, 2940, 2850, 1655, 1587, 1195, 1120, 1040, 880, 780, 700 cm^{-1}; NMR signals: τ2·1 (2H, multiplet, Ar–H) 2·5 (3H, multiplet, Ar–H), 3·30 (1H, singlet, >C=CH—), 5·90 (3H, singlet, Ar–OCH_3), 5·96 (3H, singlet, Ar–OCH_3), 6·03 (6H, singlet, Ar–OCH_3); mass spectrum: 342 (35), 328 (25), 327 (100), 299 (15), 284 (25), 267 (16), 197(20), 182(15), 102 (16). Found: C, 66·49; H, 5·35; M.W. 342·110 (high resolution mass spectrometry). $C_{19}H_{18}O_6$ required: C, 66·66; H, 5·30; M.W. 342·110.

Properties of compound D. Recrystallization was by dissolving the crude compound (0·4 g) in a minimum amount of hot $CHCl_3$ and then adding in one portion a large volume of *n*-hexane. This method yielded short colourless crystals (0·16 g after three recrystallizations), m.p. 172·0–172·5°, λ_{max} 272, 304 nm; ν(KBr): 3000, 2940, 2850, 1625, 1595, 1350, 1200, 1120, 820, 770, 690 cm^{-1}; NMR signals: τ2·22 (2H, multiplet, Ar–H), 2·59 (3H, multiplet, Ar–H), 3·28 (1H, singlet, >C=CH—), 3·42 (1H, singlet, C–8H), 6·09 (6H, singlet, Ar–OCH_3), 6·15 (3H, singlet, Ar–OCH_3); mass spectrum: 312 (25), 297(100), 295 (7), 271 (7), 269 (13), 254 (15), 167 (12), 128 (12). Found: C, 69·50, H, 4·80; M.W. 312·110 (high resolution mass spectrometry). $C_{18}H_{16}O_5$ required: C, 69·22; H, 5 16; M.W. 312·100.

Hydrolysis of compound D. Compound D (70 mg) was refluxed for 6 hr with 10 ml 50% MeOH–KOH. The reaction mixture was then cooled and neutralized with dilute HCl. Extraction with CH_2Cl_2 yielded a yellow gum (72 mg). Preparative TLC on silica gel ($CHCl_3$–10% MeOH) gave a yellow oil (24 mg) which was the least polar component. The following spectral data was obtained: λ_{max}: 281, 333 nm; ν(neat): 2940, 1610, 1110, 590 cm^{-1}; NMR signals: τ 3·50 (1H, singlet, Ar–OH), 3·75 (1H, singlet, C-5H), 6·0 (3H, singlet, Ar–OCH_3), 6·10 (3H, singlet, Ar–OCH_3), 6·25 (3H, singlet, Ar–OCH_3), 7·35 (3H, singlet, Ar–$COCH_3$); mass spectrum: 226 (72), 211(100), 195 (11), 193 (20), 183 (55), 165 (49), 151 (41). Found: C, 58·60; H, 6·14. Calc. for $C_{11}H_{14}O_5$: C, 58·40; H, 6·24.

1,2,3-*Trimethoxybenzene.* This compound, m.p. 47° (lit. m.p. 47°)[12] was prepared according to the procedure of Robinson.[12] The additional spectral data obtained is reported. λ_{max}: 267, 277 (sh) nm; ν(Nujol): 1600, 1450, 775, 735, 695 cm^{-1}; mass spectrum: 168 (100), 153 (81), 125 (49), 110 (62), 95 (47).

2,6-*Dimethoxybenzoquinone.* This compound was prepared by employing a procedure reported by Baker[13] except that the following modifications were carried out.

HNO_3 (density 1·42) was added to 1,2,3-trimethoxybenzene in ethanol at such a rate that the reaction temp. *did not* exceed 55° (about 2 hr with occasional cooling). The mixture was then stirred at 50° for a further 2 hr. Recrystallization from MeOH yielded bright yellow needles, m.p. 248·5–249° (lit. m.p. 249°),[14] λ_{max}: 285 nm; ν($CHCl_3$): 1700, 1650, 1600 cm^{-1}; NMR signals: τ 6·15 (2H, singlet, >C=CHO—), 6·75 (6H, singlet, =$COCH_3$); mass spectrum: 168 (34), 138 (15), 125 (10), 87 (11), 80 (27), 69 (100).

2,6-*Dimethoxydroquinone.* This compound, m.p. 158–160° (lit. m.p. 158°)[14] was prepared according to the procedures reported by Mauthner[14] and Baker.[13] The additional spectral data follows: λ_{max}: 285 nm; ν(Nujol): 3300, 1460, 1120 cm^{-1}; NMR signals: τ2·55 (2H, singlet, Ar–H), 6·06 (6H, singlet, Ar–OCH_3); mass spectrum: 170 (55), 168 (18), 155 (54), 127 (100), 112 (42), 109 (18), 84 (18).

2,6,-*Dimethoxyhydroquinone diacetate.* This compound, m.p. 123–124° (lit. m.p. 123°)[14] was also obtained via the procedure by Mauthner.[14] λ_{max}: 267, 275 (sh) nm; ν($CHCl_3$): 1750, 1125 cm^{-1}; NMR signals: τ 3·62 (2H, singlet, Ar–H), 6·20 (6H, singlet, Ar–OCH_3), 7·68 (3H, singlet, $ArOCOCH_3$), 7·72 (3H, singlet, $ArOCOCH_3$); mass spectrum: 254 (7), 212 (25), 170 (100), 155 (21).

Fries rearrangement of 2,6-*dimethoxyhydroquinone diacetate.* The following procedure is essentially an adaptation from that of Reynolds.[15] 2,6-Dimethoxyhydroquinone diacetate (2 g) was ground to a powder and mixed thoroughly with anhydrous $AlCl_3$ (2 g). About one-third of this mixture was added to a thick-walled flat-bottom flask which had been suspended in an oil-bath at 120–125°. After evolution of HCl gas

[12] E. CHAPMAN, A. G. PERKIN and R. ROBINSON, *J. Chem. Soc.* 3028 (1927).
[13] W. BAKER *J. Chem. Soc.* 662 (1941).
[14] F. MAUTHNER, *J. Prakt. Chem.* 287 (1936).
[15] D. REYNOLDS, J. CATHCART and J. WILLIAMS, *J. Org. Chem.* **18**, 1709 (1953).

(after 2–4 min), the mixture was stirred vigorously with a glass rod until the reaction had subsided. A second portion was added and rapid stirring was resumed. After the last portion was added the reaction mixture was stirred for 10 min. Heating with periodic stirring was continued until the evolution of HCl gas was no longer evident (about 30 min). The reaction mixture was then cooled and ground in a mortar and then added, with stirring, to a mixture of ice (2 g) and conc. HCl (1 ml). The resulting slurry was stirred for 0·5 hr, the solid was filtered and washed with cold water. After thorough drying *in vacuo* this yellow material (2 g) was examined by TLC (silica gel, $CHCl_3$) and was found to consist of 2,4-dimethoxy-3-acetoxy-6-hydroxyacetophenone with traces of the corresponding deacetylated compound.

A small portion of this mixture (25 mg) was separated by preparative TLC. The major product (20 mg, VI) was recrystallized from MeOH to yield colourless crystals, m.p. 109–110°, λ_{max}: 278, 317 nm; ν. 3520, 1760, 1620 cm^{-1}; NMR signals: τ 3·45 (1H, singlet, Ar–OH), 3 72 (1H, singlet, Ar–H), 6·10 (3H, singlet, Ar–OCH_3), 6·15 (3H, singlet, Ar-OCH_3), 7 32 (3H, singlet, Ar–$COCH_3$), 7·66 (3H, singlet, Ar–$OCOCH_3$); mass spectrum: 254 (5), 212 (100), 197 (80), 194 (15), 151 (17). Found: C, 56 61; H, 5·60. Calc for $C_{12}H_{14}O_6$: C, 56·69; H, 5·55.

2,5-*Dihydroxy*-4,6-*dimethoxyacetophenone*. The crude Fries rearrangement mixture was thoroughly dried, and then refluxed with HCl gas (5%) in MeOH for 1 hr after which time the solvent was removed. Examination of this product by TLC revealed the presence of one major component. Preparative TLC yielded yellow crystalline material, m.p. 162–163° (lit. m.p. 162–163°),[14] λ_{max}: 242, 283 nm, ν_{max} ($CHCl_3$): 3600, 1640 cm^{-1}; NMR signals: τ 3 16 (1H, singlet, Ar–OH), 3·70 (1H, singlet, Ar–H), 4·4 (1H, multiplet, Ar–OH), 6·00 (3H, singlet, Ar–OCH_3), 6·02 (3H, singlet, Ar–OCH_3), 7·30 (3H, singlet, Ar–$COCH_3$); mass spectrum: 212 (90), 197 (100), 182 (38), 179 (20), 169 (25), 151 (50). Found: C, 56·53; H, 5 68. Calc. for $C_{10}H_{12}O_5$: C, 56 60; H, 5·70.

2,3,4-*Trimethoxy*-6-*hydroxyacetophenone*. This compound was obtained via the mild methylation procedure reported by Sastri and Seshadri.[10] The resulting brown amorphous product was found to contain several components by TLC. Preparative TLC (25 mg) yielded a small amount of yellow oil (9 mg) as the least polar component. This oil was found to have spectroscopic properties identical with those of the hydrolysis product of compound D.

Acknowledgement—Financial aid from the National Research Council of Canada is gratefully acknowledged.

Phytochemistry, 1971, Vol. 10, p 3302. Pergamon Press. Printed in England

CERCIDIPHYLLACEAE

ISOLATION OF MALTOL FROM *CERCIDIPHYLLUM JAPONICUM*

KIYOKAZU TAKAISHI

Faculty of Pharmacy, Kinki University, Higashi-osaka, Osaka, Japan

(*Received* 15 *June* 1971)

Plant. Cercidiphyllum japonicum Seib. et Zucc. *Uses.* Flavour enhancer. *Isolation.* The Et_2O extract from the fresh leaves was steam distilled. The steam distillate was extracted with Et_2O and the extract was evaporated. From the residue maltol[1] (3-hydroxy-2-methyl-4-pyrone), $C_6H_6O_3$, was obtained (1·6% of dried leaves) and recrystallized from EtOAc, m.p. 162°. It was identified by m.p., mixed m.p., UV, IR and NMR.

Acknowledgement—The author thanks Dr. K. Hata of Osaka College of Pharmacy.

[1] T. KARIYONE and T. SAWADA, *Yakugaku Zasshi* **79**, 265 (1959).