Phytochemistry, Vol. 25, No. 7, pp. 1697–1699, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

PHENOLICS FROM OSAGE ORANGE WOOD CLEAVAGE OF OXYRESVERATROL

NANCY N. GERBER*

Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, P.O. Box 759, Piscataway, NJ 08854, U.S.A.

(*Received* 20 *July* 1985)

Key Word Index—*Toxylon pomiferum*; *Maclura pomifera*; Moraceae; Osage orange; phenolics; dihydrokaempferol; norartocarpenone; resveratrol; oxyresveratrol; dihydromorin.

Abstract—'Dihydromorin pentaacetate,' mp 192°, was shown to be 5,7,2',4'-tetraacetoxyflavone. Oxyresveratrol with acid or MS (probe) gave resorcinol and 7-(3,5-dihydroxyphenyl)naphthalen-1,3-diol.

INTRODUCTION

The previously known phenolic substances from Osage orange (*Toxylon pomiferum*, *Maclura pomifera*) heartwood are oxyresveratrol [1], morin [1] and dihydromorin [2]. Oxyresveratrol was thought to be responsible for the decay resistance of the wood [1] and has been shown to inhibit the growth of six dermatophytes [3]. Recently, the water-insoluble fractions from our original Osage orange heartwood extractions [1] were re-examined using TLC and the solvent systems employed in the investigations of the heartwoods of five *Morus* species [4]. In addition to dihydromorin, three phenolic substances not previously reported from Osage orange heartwood were obtained in pure form: dihydrokaempferol, norartocarpenone and resveratrol. They were identified by their R_f values relative to authentic samples of the previously known phenolic compounds and by UV, ^{1}H NMR and MS. Reservatrol [5] and dihydrokaempferol [6] were previously known from several sources. Norartocarpenone was recently isolated from *M. rubra* [4].

The characterization of these three substances makes a total of six phenolics which have been isolated both from Osage orange wood and various *Morus* sp. [4, 7]. This suggests a closer taxonomic relationship between these two than between them and other Moraceae (e.g. *Artocarpus*, *Chlorophora*) especially since isoprenoid substituted flavanoids and stilbenes were absent in both cases.

RESULTS AND DISCUSSION

We obtained the known acetate derivative of dihydromorin, 'dihydromorin pentaacetate' (mp 192–195°; lit. mp 191° [4], 190–193° [8], 192° [2], 190–194° [9]) but the ^{1}H NMR spectrum clearly showed the presence of four acetoxy groups not five (3H each at δ 2.05 and 2.43, 6H at 2.33). Furthermore the AB pattern characteristic of the *trans* hydrogens at C-2 and C-3 which was seen in the ^{1}H NMR spectra of dihydromorin, dihydrokaempferol and its tetraacetate derivative was replaced by a one proton singlet band at δ 5.8. Since the MS of dihydromorin showed the expected $[M]^+$ at m/z 304 ($C_{15}H_{12}O_7$), clearly dehydration of the hydroxy group at C-3 had occurred during preparation of the acetate so that the 192° acetate is 5,7,2',4'-tetraacetoxyflavone. The corresponding tetrahydroxyflavone has been isolated from *Artocarpus heterophyllus* [10, 11] but the acetate was not prepared. The ^{1}H NMR spectrum of Laidlaw's 'dihydromorin pentaacetate' [2, 8] had been examined [12] along with those of other 2,3-dihydroflavones but the authors reported only chemical shifts of the heterocyclic ring protons, no integration data, and explained the δ5.73 singlet in 'dihydromorin pentaacetate' as an equivalence of the protons at C-2 and C-3. The facile dehydration suggests that the stereochemistry at C-2 and C-3 in dihydromorin might differ from that of other dihydroflavones which cannot be dehydrated even under drastic conditions [6]. However, in view of the identical coupling constants ($J_{2,3}$ = 11.5 Hz) shown by dihydromorin and dihydrokaempferol this seems unlikely.

In 1954, we were unsuccessful in attempts to regenerate oxyresveratrol using acids from either its tetraacetate or tetramethyl ether [1]. The experiments gave resorcinol and another (presumed) fragment isolated as an acetate, mp 151–153°, λ_{max} 253, 294. Since with MS and ^{1}H NMR this substance might now be readily identified, the direct acid treatment of oxyresveratrol was undertaken. Resorcinol and the 151° acetate were again obtained. The acetate showed $[M]^+$ at m/z 436 and fragmentation ions due to loss of one, two, three and four CH_2CO units. The ^{1}H NMR spectrum showed in addition to four acetate groups (9H at δ2.33, 3H, at 2.5) only a complex pattern due to eight hydrogens in the aromatic region, δ6.9–8.2. Thus the acetate was shown to be $C_{16}H_8(OOCMe)_4$; the parent substance was therefore $C_{16}H_{12}O_4$, and a rational structure for it is the previously unreported 7-(3,5-dihydroxyphenyl)naphthalen-1-3-diol. The behavior of oxyresveratrol in aqueous acid is then readily understood as the hydration of the double bond and a reverse aldol reaction followed by dimerization of the 3,5-dihydroxyphenylacetaldehyde. This dimerization has ample precedent in the synthesis of 2-phenylnaphthalene from phenylacetaldehyde [13, 14], 2-*p*-tolyl-7-methylnaphthalene from *β*-bromo-*p*-methylstyrene [15] and 2-(3,4-methylene dioxyphenyl)-6,7-methylene-dioxynaphthalene from homopiperonal [16].

The MS of resveratrol showed $[M]^+$ at m/z 228 as the base peak. A carefully purified sample of oxyresveratrol always gave two peaks in the total ion current graph as if it

*Deceased April 1985.

contained a second component. The MS of the more volatile component was that expected from oxyresveratrol with $[M]^+$ at m/z 244 as the base peak. The second component furnished essentially two ions, at 268 $[M]^+$ (30%) and 110 $[C_6H_5O_2]^+$ (100%), and is explained as the 7-(3,5-dihydroxyphenyl)naphthalen-1,3-diol formed in the direct inlet probe by heat and electron impact. Oxyresveratrol at 100 μg/ml and resveratrol at 120 μg/ml were inactive against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

EXPERIMENTAL

Acetylations were carried out at room temp. overnight using excess Ac_2O and a trace of pyridine. For ^1H NMR, at 60 MHz with TMS, free hydroxystilbenes and flavones were dissolved in Me_2CO and acetate derivatives in $CDCl_3$. MS were obtained with the Hewlett–Packard 5985, a quadrople instrument, using the electron impact, direct inlet mode and an accelerating voltage of 70 eV.

Separations. Separations were carried out by CC on MN cellulose powder for column chromatography eluting with 30% aq. HOAc and on Applied Science Hi-Flosil 60/200 mesh eluting with C_6H_6–Me_2CO (75:10). About 100 g of cellulose or 15 g of silica were used for the separation of 1 g of a mixture. Cellulose columns were washed clean and re-used.

All fractions were monitored by TLC on EM pre-coated TLC plastic sheets of cellulose F_{254} in 30% aq. HOAc and on MN Polygram sil G UV_{254} in C_6H_6–Me_2CO (70:30). The fluorescence quenching substances (dihydromorin, dihydrokaempferol and norartorarpenone) were most easily detected at low concns on the silica plates. The fluorescent substances (morin, resveratrol and oxyresveratrol) were more easily visible on the cellulose plate.

Dihydromorin. Mp 223–225° (dec.) (lit. [4] mp 228°); UV λ_{max} 291 nm; R_f 0.7 (cellulose), 0.4 (silica); ^1H NMR: δ12.0 (1H, s, C-5 OH), 6.13 and 6.18 (2H, both *d*, J = 2 Hz, H-6 and H-8), 5.0 and 5.67 (2H, both *d*, J = 11.5 Hz, H-2 and H-3), 6.5–6.75 (2H, *m*), 7.53 (1H, *d*, J = 9 Hz, H-3', H-6' and H-5'). MS m/z (rel. int.): 304, $[M]^+$ (40), 286 $[M-18]^+$ (15), 275 $[M-29]^+$ (45), 257 $[M-29-18]^+$ (13), 153 (100), 150 (28), 123 (45). Acetate derivative: mp and ^1H NMR in discussion except for δ6.75 and 6.9 (2H, both *d*, J = 2 Hz, H-6 and H-8).

Dihydrokaempferol. Mp 225–230° (lit. [4] mp 228–230°) UV λ_{max} 293 nm; R_f 0.7 (cellulose), 0.6 (silica); ^1H NMR: δ12.0 (1H, s, C-5 OH), 6.12 and 6.17 (2H, both *d*, J = 2 Hz, H-6 and H-8), 5.28 and 4.78 (2H, both *d*, J = 11.5 Hz, H-2 and H-3), 7.0–7.3 and 7.5–7.8 (4H, *m*, H-2', H-3', H-5', H-6'). MS m/z (rel. int.): 288 $[M]^+$ (54), 270 $[M-18]^+$ (12), 260 (13), 259 $[M-29]^+$ (80), 165 (27), 153 (100), 136 (33), 134 (48), 107 (76). Acetate derivative: amorphous, softens 80–85°; ^1H NMR: δ2.05 (3H, s, OAc), 2.35 (6H, s, OAc), 2.43 (3H, s, OAc), 6.78 and 6.97 (2H, both *d*, J = 2 Hz, H-6 and H-8), 5.53 and 5.9 (2H, both *d*, J = 12 Hz, H-2 and H-3).

Norartocarpenone. Mp 225–230° (dec.) (lit. [4] mp 233–234° dec.); UV λ_{max} 295 nm; R_f 0.7 (cellulose), 0.5 (silica). ^1H NMR: δ12.5 (1H, s, C-5 OH), 6.13 (2H, s, H-6 and H-8), 5.85 (1H, *dd*, J = 13.4 Hz, H-2), 3.2 (in pyridine, 2H, *m*, H-3), 6.5–7.7 (3H, *m*, H-3', H-5' and H-6'). MS m/z (rel. int.): 288 $[M]^+$ (27), 271 (18), 270 $[M-18]^+$ (100), 269 (63), 259 $[M-29]^+$ (17), 153 (54), 136 (13), 134 (10), 107 (19). Acetate derivative, glass (lit. [4] mp 105–106°). ^1H NMR δ2.03 (3H, s, OAc), 2.35 (6H, s, OAc), 2.40 (3H, s, OAc), 2.95 (2H, *m*, C-3); 1H (H-2, *dd*) not visible because of small sample.

Resveratrol. Mp 256–260° (lit. [4] mp 255–257°); UV λ_{max} 305, 320 nm. R_f 0.3 (cellulose), 0.5 (silica). MS m/z (rel. int.): 228 $[M]^+$ (100), 211 $[M-17]^+$ (12), 181 (18). Acetate derivative mp 108–112°. ^1H NMR: δ2.3 (9H, s, OAc), 6.7–7.6 (9H, m).

Oxyresveratrol. UV λ_{max} 310, 323 nm. R_f 0.2 (cellulose), 0.3 (silica). MS m/z (rel. int.): 244 $[M]^+$ (100), 227 $[M-17]^+$ (25), 226 $[M-18]^+$ (39), 198 (22), 197 (20), 123 (44). Acetate derivative mp 132–137° (lit. [4] mp 142–143°). ^1H NMR: δ2.30 (9H, s, OAc), 2.37 (3H, s, OAc), 6.8–7.8 (8H, *m*).

Acid cleavage of 2,3',4,5'-tetramethoxystilbene (1953). A mixture of HOAc (1 ml) constant-boiling HI (1 ml) and the tetramethoxystilbene (100 mg) was refluxed 0.5 hr, poured into 5% aq. $NaHCO_3$ (50 ml) and the resulting soln extracted with Et_2O. The dried Et_2O soln was concd and chromatrographed on a 5.0 g silicic acid column. Elution with Et_2O until a yellow band reached the bottom of the column furnished crystalline resorcinol, 32 mg (93%) which after recrystallization from iso-octane, melted at 109–110° undepressed when mixed with an authentic sample. Further elution of the column with Et_2O furnished a brown solid (34 mg) which was acetylated. The brown crystalline acetate isolated in the usual way was chromatographed in C_6H_6 on 3 g silicic acid column. Elution with 5% Et_2O in C_6H_6 furnished a colourless oil (20 mg) which was crystallized from C_6H_6–iso-octane, mp 151–153°; UV λ_{max} 253, 294 nm. It was insoluble in aq. $NaHCO_3$, gave no precipitation with 2,4-dinitrophenylhydrazine reagent and did not immediately decolourize $KMnO_4$ in Me_2CO.

Acid cleavage of oxyresveratrol. A mixture of oxyresveratrol (50 mg), HOAc (3 ml) and conc HCl (2 ml) was heated 30 min on the steam bath, then poured into 50 ml of 5% aq. $NaHCO_3$. The resulting soln (pH 4.5) was extracted immediately with EtOAc. TLC of the extract on silica disclosed resorcinol at R_f 0.4 and a UV-absorbing main spot at R_f 0.2 which rapidly darkened in air. This substance was isolated by CC on pH 4 silica (Mallinkrodt SilicAR-4 100–200 mesh) eluting with increasing concns of Me_2CO in C_6H_6 then immediately acetylated. The acetate, 13 mg, mp 148–151°, was obtained by filtration. MS m/z (rel. int.): 436 $[M]^+$ (7), 394 $[M-Ac]^+$ (46), 352 $[M-2Ac]^+$ (100), 310 $[M-3Ac]^+$ (54), 268 $[M-4Ac]^+$ (62), 239 $[268-29]^+$ (22). ^1H NMR: δ2.33 (9H, s, OAc), 2.5 (3H, s, OAc), 6.9–8.2 (8H, *m*).

Acknowledgements—I thank H. A. Lechevalier for antimicrobial testing. This work was partially supported by the Charles and Johanna Busch Memorial Fund.

REFERENCES

1. Barnes, R. A. and Gerber, N. N. (1955) *J. Am. Chem. Soc.* **77**, 3259.
2. Laidlaw, R. A. and Smith, G. A. (1959) *Chem. Ind. (London)* 1604.
3. Ratanabanangkoon, K., Poopyruchpong, N. and Charoensiri, K. (1976) *J. Sci. Soc. Thailand* **2**, 202.
4. Deshpande, V. H., Srinivasan, R. and Rama Rao, A. V. (1975) *Indian J. Chem.* **13**, 453.
5. Billek, G. (1964) *Fortsch. Chem. Org. Nat. Prod.* **22**, 115. Langcake, P. and Pryce, R. J. (1976) *Physiol. Plant Pathol.* **9**, 77.
6. Geissman, T. A. (ed.) (1962) *The Chemistry of Flavanoid Compounds*, p. 452. Macmillan, New York.
7. Paris, R., Debray, M. and Etchepare, S. (1966) *Ann. Pharm. Fr.* **24**, 745.
8. Carruthers, W. R., Farmer, R. H. and Laidlaw, R. A. (1957) *J. Chem. Soc.* 4440.
9. Chakravarty, B. and Seshadri, T. P. (1962) *Tetrahedron Letters* 787.
10. Radhakrishna, P. V. and Rama R/o, A. V. (1965) *Tetrahedron Letters* 633.

11. Radhakrishna, P. V. and Rama Rao, A. V. (1966) *Indian J. Chem.* **4**, 406.
12. Clark-Lewis, J. W., Jackman, L. M. and Spotswood, T. M. (1964) *Aust. J. Chem.* **17**, 632.
13. Bailey, A. S. and Case, R. J. (1958) *Tetrahedron Letters* **3**, 129.
14. Zincke, X. and Breuer, X. (1884) *Annalen* **220**, 24.
15. Auwers, K. and Keil, G. (1903) *Chem. Ber.* **36**, 3902.
16. Erdtman, H. and Robinson, R. (1930) *J. Chem. Soc.* 1530.