PLANT ANTICANCER AGENTS. XXVI. CONSTITUENTS OF *PEDDIEA FISCHERI*[1]

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ABSTRACT.—The *in vitro* P-388 lymphocytic leukemia activity of the chloroform-soluble fraction of the roots of *Peddiea fischeri* (Thymelaceaceae) was found to be due principally to the quinone 2,6-dimethoxybenzoquinone (2,6-DMBQ), and to a lesser extent the two coumarins daphnoretin and umbelliferone. This is the first report of the occurrence of 2,6-DMBQ in the family Thymelaeaceae. The occurrence of 2,6-DMBQ in higher plants is reviewed.

In a continuing search for antineoplastic agents from terrestrial plants, the roots of *Peddiea fischeri* Engl. (Thymelaeaceae) were investigated. There have been no phytochemical or biological studies reported on this genus.

In the present investigation, the chloroform extract of the roots displayed activity in the P-388 lymphocytic leukemia test system *in vitro* (ED_{50} 0.038 μg/ml)[3]. Bioactivity-directed fractionation of this extract led to the isolation of daphnoretin, umbelliferone and 2,6-dimethoxybenzoquinone.

EXPERIMENTAL[4]

PLANT MATERIAL.—Roots of *Peddiea fischeri* Engl. (Thymelaeaceae) were collected in Kenya in 1979 by the Economic Botany Laboratory, BARC-East, Beltsville, MD. A voucher specimen documenting the collection is deposited in the Herbarium of the National Arboretum, Washington, D.C.

EXTRACTION AND FRACTIONATION.—Extraction of the air-dried and milled roots (27 kg) of *P. fischeri* with petroleum ether (bp 60–80°) afforded a petroleum ether extract (145 g, P-388, ED_{50} 0.21 μg/ml). The remaining marc was extracted with chloroform, and evaporation *in vacuo* afforded a chloroform extract (160 g, P-388, ED_{50} 0.038 μg/ml). Partition between between petroleum ether and methanol:water (9:1) yielded a petroleum ether-soluble fraction (35 g, P-388, ED_{50} 0.25 μg/ml), and further extraction with chloroform gave a chloroform-soluble fraction (50 g) and a chloroform-insoluble fraction (14.5 g). Only the chloroform-soluble fraction was further examined.

CHROMATOGRAPHIC SEPARATION OF THE CHLOROFORM-SOLUBLE FRACTION.—The chlorform-soluble fraction (50 g) was chromatographed on silica gel[5] (750 g) and eluted with chloroform and chloroform-methanol mixtures of increasing polarity. About 30 fractions of one liter each were collected. Fraction 4, eluted with chloroform-1% methanol (P-388, ED_{50} 0.53 μg/ml), yielded daphnoretin (1.45 g, 0.0048%), which crystallized from chloroform/methanol, mp 234°; ir, νmax (KBr) 3400, 1730, 1620, 1590, 1510 and 1280 cm^{-1}; uv, λmax (MeOH) 228 (log ϵ 4.21), 260 (3.60) and 340 nm (4.20); ^{1}H nmr, (DMSO-d_6, 60 MHz) δ 3.82 (3H, s, $ArOCH_3$), 6.40 (1H, d, J=9.8 Hz, 3'-H), 7.15 (1H, d, J=8.7 Hz, 6'-H), 7.30 (1H, d, J=8.7 Hz, 5'-H), 6.95, 7.70, 7.90 and 7.98 (1H each, s, 4 x Ar-H) and 8.10 (1H, d, J=9.8 Hz, 4'-H); ms, m/z 352 (M^+, 100%), 324 (5), 309 (12), 294 (4), 270 (5), 191 (3), 179 (52), 164 (14), 145 (10), 120 (4), 117 (12), 89 (58), 68 (23), 62 (37) and 50 (12). These spectral data are consistent with those reported (3) for daphnoretin.

Further elution of the column with the same solvent system afforded an *in vitro* active

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[3]All extracts, fractions and pure compounds were bioassayed according to established protocols (2). An extract, fraction or pure compound is considered active if it displays T/C$\geq$ 125% *in vivo* (P-388 test system), and an isolate is considered active if it shows an $ED_{50} \leq 4$ μg/ml in the KB or P-388 cell culture system *in vitro*.

[4]Melting points were determined by means of a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman, model DB-G, grating spectrophotometer, and the ir spectra with a Beckman, model IR-18A, spectrophotometer. Proton magnetic resonance spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions with a Varian model T-60A instrument, operating at 60 MHz with a Nicolet, model TT-7, Fourier Transform attachment. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ units. Mass spectra were obtained with a Varian MAT 112S, double focusing spectrometer operating at 70 eV.

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fraction (P-388, ED_{50} <0.01 μg/ml) from which umbelliferone (200 mg, 0.00066%) was isolated, mp 235–237°; ir, νmax (KBr) 3160, 1725, 1625, 1695 and 1600 cm^{-1}; ^1H nmr, (DMSO-d_6, 60 MHz) δ 6.20 (1H, d, J=9.4 Hz, 3-H), 6.73 (1H, d, J=2.5 Hz, 8-H), 6.76 (1H, m, 6-H), 7.53 (1H, d, J=8.3 Hz, 5-H) and 7.93 (1H, d, J=9.4 Hz, 4-H); ms, m/z (M^+, 100%), 134 (89), 106 (8), 105 (21), 78 (29), 77 (15), 69 (10), 67 (15), 62 (11) and 51 (22). These spectral data are consistent with those reported (3) for umbelliferone.

The fraction eluted from the column with chloroform (2 g, P-388, ED_{50} 0.098 μg/ml) was rechromatographed on silica gel (40 g) and eluted with chloroform. The first fraction afforded 2,6-dimethoxybenzoquinone (20 mg, 0.00006%), mp 195–198°; ir, νmax (KBr) 1710, 1660, 1615 and 1490 cm^{-1}; uv, λmax (MeOH) 288 nm (log ε 4.10); ^1H nmr ($CDCl_3$, 60 MHz) δ 3.82 (6H, s, 2 x OCH_3) and 5.85 (2H, s, 3,5-H]); ms, m/z 168 (M^+, 52%), 140 (8), 138 (16), 125 (11), 112 (10), 110 (7), 97 (11), 80 (38) and 69 (100). Comparison with 2,6-dimethoxybenzoquinone obtained previously (4) confirmed the identity.

Biological activity of the isolates.—Daphnoretin (NSC-291852), umbelliferone (NSC-019790), and 2,6-dimethoxybenzoquinone (NSC-56336) displayed activities of 2.2, 0.4 and 0.0015 μg/ml, respectively, in the P-388 lymphocytic leukemia test system *in vitro*. Daphnoretin and umbelliferone were also evaluated at doses in the range 2.2–17.5 mg/kg and 1.4–11.4 mg/kg, respectively, in the P-388 test system *in vivo* but were found to be inactive.

DISCUSSION

We could find no reports of any ethnomedical information, biological testing *in vitro*, *in vivo*, or in humans, nor any reports of prior phytochemical studies on the genus *Peddiea*.

Fractionation of the chloroform extract of *Peddiea fischeri* native to Kenya by means of the P-388 lymphocytic leukemia test system *in vitro* afforded 2,6-dimethoxybenzoquinone as the most active constituent. The first report of the cytotoxicity of 2,6-DMBQ in the KB test system *in vitro* (4) was subsequently confirmed by Kingston and co-workers (5). This present report indicates that 2,6-DMBQ is approximately 100 times more active in the P-388 system. Unfortunately, insufficient material was available for *in vivo* testing; such experiments might yield interesting results.

A surprisingly widespread compound, 2,6-DMBQ has been reported in 46 genera in 27 families, exclusive of the present report, as shown in Table 1. The

Table 1. Distribution of 2,6-dimethoxybenzoquinone in higher plants.

Family	Genus (Reference)
Aceraceae	*Acer* (6)
Apocynaceae	*Rauvolfia* (7)
Asclepiadaceae	*Marsdenia* (8)
Betulaceae	*Betula* (9)
Compositae	*Verbesina* (10)
Dipterocarpaceae	*Shorea* (11)
Ericaceae	*Enkianthus* (12), *Tripetalia* (13)
Fagaceae	*Fagus* (14), *Quercus* (11,15)
Flacourtiaceae	*Xylosma* (4)
Gramineae	*Sasa* (16), *Triticum* (17)
Guttiferae	*Kielmeyera* (18)
Leguminosae	*Acacia* (11,19), *Afzelia* (11), *Bowdichia* (20), *Caesalpinia* (21), *Glycine* (22), *Guibortia* (11), *Milletia* (11,19), *Pericopsis* (11), *Phaseolus* (22)
Magnoliaceae	*Liriodendron* (11), *Talauma* (11)
Melastomatoceae	*Tibouchina* (5)
Meliaceae	*Cedrela* (11), *Entandrophragma* (11,23), *Khaya* (11,24), *Swietenia* (11,19)
Moraceae	*Brosimum* (11)
Myristicaceae	*Virola* (11)
Proteaceae	*Hakea* (25)
Ranunculaceae	*Adonis* (26)
Rhizophoraceae	*Kandelia* (27)
Rubiaceae	*Canthium* (28)
Salicaceae	*Populus* (29)
Sapotaceae	*Tieghemella* (11,19)
Simaroubaceae	*Ailanthus* (30–33), *Eurycoma* (23), *Picrasma* (24,34), *Quassia* (34), *Samadera* (35), *Simarouba* (34)
Ulmaceae	*Ulmus* (36,37)
Vochysiaceae	*Vochysia* (38)
Zingiberaceae	*Aframomum* (39)

significant cytotoxicity of this compound should, therefore, be considered in relation to fractionation of highly cytotoxic (P-388 test system) non-alkaloidal fractions.

As expected, daphnoretin and umbelliferone, which were substantially less cytotoxic, showed no *in vivo* activity at doses up to 17.5 and 11.4 mg/kg, respectively. This is in agreement with previously determined data (40,41).

It is noteworthy that none of the phorbol or daphnane esters which are frequently found as antineoplastic constituents in members of this plant family were isolated in this study.

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LITERATURE CITED

1. S. S. Handa, A. D. Kinghorn, G. A. Cordell and N. R. Farnsworth, *J. Nat. Prod.*, in press.
2. R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rept. Pt. 3*, **3**(2), 1 (1972).
3. M. Williams and J. M. Cassady, *J. Pharm. Sci.* **65**, 912 (1976).
4. G. A. Cordell, P. T. O. Chang, H. H. S. Fong and N. R. Farnsworth, *Lloydia*, **40**, 340 (1977).
5. E. Jones, O. Ekundayo and D. G. I. Kingston, *J. Nat. Prod.*, **44**, 493 (982).
6. V. J. Filipic and J. C. Underwood, *J. Food Sci.*, **29**, 464 (1964).
7. S. M. Kupchan and M. E. Obasi, *J. Amer. Pharm. Assoc.* **49**, 257 (1960).
8. K. Ito and J. Lai, *Yakugaku Zasshi*, **98**, 1285 (1978).
9. M. Yokota, H. Zenda, T. Kosuge, T. Yamamoto and Y. Torigoe, *Yakugaku Zasshi*, **98**, 1607 (1978).
10. W. Herz and N. Kumar, *Phytochemistry*, **20**, 247 (1981).
11. B. M. Hausen, *Contact Dermatitis*, **4**, 204 (1978).
12. M. Ogawa, S. Hisada and I. Inagaka, *Yakugaku Zasshi*, **92**, 1395 (1973).
13. M. Yasue, J. Sakakibara, H. Ina and I. Yanagisawa, *Yakugaku Zasshi*, **94**, 1634 (1973).
14. K. Freudenberg and G. S. Sidhu, *Holzforschung* **15**, 33 (1961).
15. M. K. Seikel, F. D. Hostettler and G. J. Niemann, *Phytochemistry*, **10**, 2249 (1971).
16. K. Yamagashi, S. Yasuda and M. Honzawa, *Mokuzai Gokkaishi*, **18**, 131 (1972).
17. L. Vuatez, *Helv. Chim. Acta*, **33**, 433 (1950).
18. D. de Barros Correa, L. G. Fonseca de Silva, O. R. Gottlieb and S. Janot Goncalves, *Phytochemistry*, **9**, 447 (1970).
19. H. Schmalle, O. Jarchow and B. M. Hausen, *Naturwissenschaften*, **64**, 534 (1977).
20. P. M. Brown, R. H. Thompson and B. M. Hausen, *Justus Liebigs Ann. Chem.* 1295 (1974).
21. D. D. McPherson, G. A. Cordell, J. M. Pezzuto and H. H. S. Fong, unpublished results.
22. J. Berlin and W. Barz, *Planta Med. Suppl.* 134 (1975).
23. Le Van Thoi and Nguyen-Ngoc-Suong, *J. Org. Chem.*, **35**, 1104 (1970).
24. J. Polonsky and E. Lederer, *Bull. Soc. Chim. Fr.*, 1157 (1959).
25. M. Varma, R. S. Varma and M. R. Parthasarathy, *Z. Naturforsch.*, **35C**, 344 (1980).
26. W. Karrer, *Helv. Chim. Acta*, **13**, 1424 (1930).
27. A. Kato and J. Takahashi, *Phytochemistry* **15**, 2028 (1976).
28. L. H. Briggs, R. C. Cambie and E. F. Orgia, *N.Z.J. Sci.*, **21**, 377 (1978).
29. I. A. Pearl and S. F. Darling, *Phytochemistry*, **7**, 1851 (1968).
30. C. G. Casinovi and G. Grandolini, *Atti Accad. Noz. Licei, Cl. Sci. Fis. Mat. Nat. Rend.*, **35**, 348 (1963).
31. M. K. Jain, *Indian J. Chem.*, **2**, 40 (1964).
32. J. Polonsky and J. L. Fourrey, *Tetrahedron Lett*, 3983 (1964).
33. M. Ogura, G. A. Cordell, A. D. Kinghorn and N. R. Farnsworth, *Lloydia*, **40**, 579 (1977).
34. N. Inamoto, S. Masuda, O. Simamura and T. Tsuyuki, *Bull. Chem. Soc. Japan*, **34**, 888 (1961).
35. J. Polonsky, J. Zybler and R. O. B. Wijesekera, *Bull. Soc. Chim. Fr.*, 1715 (1962).
36. F. D. Hostettler and M. K. Seikel, *Tetrahedron*, **25**, 2325 (1969).
37. M. Lehrer and R. Stevenson, *J. Chem. Soc., Perkin Trans I* 1165 (1974).
38. D. De Barros Correa, E. Birchal, J. E. V. Aguilar and O. R. Gottlieb, *Phytochemistry*, **14**, 1138 (1975).
39. G. Vidari, P. V. Finzi and M. de Bernardi, *Phytochemistry*, **10**, 3335 (1971).
40. M. Suffness, Natural Products Branch, NCI, private communication.
41. S. J. Torrance, J. J. Hoffmann and J. R. Cole, *J. Pharm. Sci.*, **68**, 664 (1979).