In conclusion, it can be stated that the presence of elemicin in one sample was the most striking difference noticed on comparison of the essential oil samples analyzed. Because of the high content of eugenol in some of the samples, the oil of *O. trichodon* might serve as a valuable source of that compound, which is used in dentistry.

ACKNOWLEDGMENTS

We wish to record our thanks to Mr. F.X. Ayobangira, Curphametra, for identification of the plant material; to Dr. W. Onkenhout for recording the mass spectra; to Dr. H. Hendriks, State University of Groningen, the Netherlands; Prof. Dr. H. Jork, University of Saarland, Saarbrücken, FRG; and Dr. A.J.A. van der Weerdt, Naarden International, Naarden, the Netherlands), for reference substances; to Curphametra, Butare, Rwanda), for supporting one of the authors (LN) for a study stay in Leiden; to Mr. W. Teller, Office for International Cooperation, Leiden University, the Netherlands, for administrative support; and to the European Development Fund, European Community, Brussels, Belgium, for a grant (to LN).

LITERATURE CITED

1. "Flore du Rwanda, Spermatophytes." Ed. by G. Troupin, Vol. III, Butare (Rwanda), INRS publication No. 30, 1985, pp. 321-327.
2. F.X. Ayobangira, L. Ntezurubanza, F.X. Habiyaremye, and L. van Puyvelde, *Bull. Agric. Rwanda,* **18**, 110 (1985).
3. L. Ntezurubanza, R. Panebianco, and F.X Ayobangira, *Bull. Agric. Rwanda,* **18**, 187 (1985).
4. R. Hegnauer, "Chemotaxonomie der Pflanzen," Vol. IV, Birkhäuser Verlag, Basel/Stuttgart, 1966, pp. 313-314.
5. J.J.C. Scheffer, A. Koedam, and A. Baerheim Svendsen, *Chromatographia,* **9**, 425 (1976).
6. J.J.C. Scheffer, A. Gani, and A. Baerheim Svendsen, *Sci. Pharm.,* **49**, 337 (1981).
7. J.J.C. Scheffer, A. Koedam, and A. Baerheim Svendsen, *Medd. Nor. Farm. Selsk.,* **39**, 161 (1977).
8. L. Ntezurubanza, J.J.C. Scheffer, A. Looman, and A. Baerheim Svendsen, *Planta Med.,* **50**, 385 (1984).
9. S.N. Sobti, M.K. Khosla, P. Pushpangadan, and R.K. Thapa, *Herba Hung.,* **20** (1-2), 49 (1981).

*Received 1 May 1986*

## METABOLITES OF *NECTRIA FUCKELIANA*

WILLIAM A. AYER and LISA M. SHEWCHUK

*Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*

*Nectria fuckeliana* Booth (Pyrenomycetes) was isolated from Douglas fir near Lumby, B.C., by A. Funk, Pacific Forest Research Center, Canadian Forestry Service, Victoria, British Columbia. The fungus is a wound parasite sometimes associated with dieback but most frequently found on damaged, decaying logs (1). We became interested in the metabolites produced by this fungus when Funk observed that on several occasions, orange-red, needle-like crystals appeared on the surface of potato dextrose agar cultures of *N. fuckeliana*. We report herein the isolation and identification of two of these metabolites.

EXPERIMENTAL

A culture of *N. fuckeliana* PFRC 537 was obtained from A. Funk and deposited in the University of Alberta Microfungus Collection (accession number UAMH 5025).

PREPARATION OF FUNGUS.—The fungus was grown on solid media (potato dextrose agar, 20°, 4-6 weeks) or in liquid still culture (malt extract media, 24°, 10 months).

EXTRACTION, ISOLATION, AND IDENTIFICATION.—The aqueous broth (10 liters) was extracted with EtOAc (48 h). Concentration of the extract gave a mixture of products (0.28 g). Flash chromatography on silica gel and elution with $Et_2O$-Skellysolve B (1:20) afforded 2,5-dimethoxy-3,6-dimethylbenzoquinone (9.4 mg) and (−)-mellein (6.1 mg). The orange-red crystals collected from solid cultures of *N. fuckeliana* were shown to be 2,5-dimethoxy-3,6-dimethylbenzoquinone. The compounds were identified by comparison of their physical (mp) and spectral (ir, nmr, ms, uv) properties with those reported in the literature (2,3).

The metabolites produced by several species of *Nectria* have been reported previously (4). 2,5-Dimethoxy-3,6-dimethylbenzoquinone is a known metabolite of *Nectaria coryli* (2), whereas this is the first reported isolation of (−)-mellein from a species of this genus.

## LITERATURE CITED

1. A. Funk, in: "Parasitic Microfungi of Western Trees," Canadian Forestry Service Publication BC-X-222, Victoria, B.C., 1981, p. 97.
2. M.S.R. Nair and M. Anchel, *Tetrahedron Lett.*, 795 (1972).
3. M. Matsui, K. Mori, S. Araski, *Agr. Biol. Chem.*, **12**, 896 (1964).
4. W.B. Turner, and D.C. Aldridge, in: "Fungal Metabolites II," Academic Press, New York, 1983.

*Received 8 May 1986*

# COUMARINS FROM BARK OF *AMYRIS BARBATA*

CARLOS HASBUN*

*Department of Chemistry, National University, Heredia, Costa Rica 3000*

OSCAR CASTRO

*Center for the Investigation of Natural Products (CIPRONA), School of Chemistry, University of Costa Rica, San Jose, Costa Rica 2060*

Species of *Amyris* (Rutaceae) are known to be rich sources of coumarins, and recently some quinoline alkaloids and novel nicotinamide derivatives have been reported (1,2). *Amyris barbata* Lundell is a tree that grows in the premontane forest of Costa Rica, constituting a precious wood for its durability and resistance. It has been used in traditional medicine for the treatment of skin diseases (3)[1]. We have examined the bark of *A. barbata* and report, in addition to β-sitosterol and *p*-anisic acid, three known coumarins: scopoletin, imperatorin, and oxyimperatorin. The compounds were characterized by comparison with literature data (4-6) or with authentic samples.

## EXPERIMENTAL

PLANT MATERIAL.—The material was collected in December in Santa Ana, Province of San Jose, Costa Rica, and identified by L.J. Poveda; voucher specimens have been deposited at the Herbarium of the National Museum, No. 108523. Uv spectra were recorded in MeOH; $^1$H-nmr and $^{13}$C-nmr spectra were recorded with TMS as internal standard in $CDCl_3$. Si gel 60 (Merck, 0.040-0.060 mm) was used for column chromatography and Si gel G (Merck) for tlc.

ISOLATION OF CHEMICAL CONSTITUENTS.—Bark of *A. barbata* (1.47 kg) was extracted with MeOH at room temperature and concentrated under reduced pressure to yield a crude extract (52 g), which was partitioned with hexane (20.6 g) and EtOAc (5.17 g). The concentrated hexane extract (10 g) was fractionated by flash chromatography (500 g Si gel, 50 ml fractions); 100 fractions eluted with hexane-$Et_2O$ (1:1) were collected, and each was analyzed by tlc. Fractions 17-32, after successive recrystallizations from MeOH, afforded β-sitosterol (0.50 g) identified by standard sample comparison (mmp, ir, $^1$H nmr, tlc). Fractions 89-100 were combined and subjected to preparative tlc on Si gel using hexane-EtOAc (3:1), which yielded imperatorin (0.02 g). The EtOAc concentrate (5.17 g) was fractionated by flash chromatography (300 g Si gel, 25 ml fractions) into 82 fractions using hexane-EtOAc (3:1) as eluent. Fractions 11-48 were rechromatographed over Si gel with hexane-EtOAc (3:1) which yielded scopoletin (0.33 g, $^1$H nmr, $^{13}$C nmr, mp) (7) and imperatorin (0.70 g, uv, $^1$H nmr, mp) (8, 9). Fractions 49-82 were purified by using the same chromatography system as above but with $CHCl_3$-MeOH (95:5) as eluent to give imperatorin oxide (0.61 g, $^1$H nmr, $^{13}$C nmr, mp) (10), scopoletin (0.05 g), and *p*-anisic acid (0.015 g), which was identified by mp, co-tlc and superposable ir with an authentic sample obtained by treating *p*-anisic-acid methyl ester with base (NaOH 1N) at room temperature for 1 h. Details of the identification are available from the major author.

[1]L.J. Poveda, personal communication, 1985.