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J. Nat. Prod., **1994**, 57 (10), 1411-1415• DOI: 10.1021/np50112a010 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 10, pp. 1411-1415, October 1994

ELLAGITANNINS AND COMPLEX TANNINS FROM *QUERCUS PETRAEA* BARK

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ABSTRACT.—The ellagitannins 2,3-(*S*)-hexahydroxydiphenoyl-glucose, pedunculagin, vescalagin, and castalagin; the flavanoellagitannins acutissimin A, acutissimin B, eugenigrandin A, guajavin B, and stenophyllanin C; and the procyanidinoellagitannin mongolicanin have been isolated from the bark of *Quercus petraea*. The ellagitannin fraction had a weak antisecretory effect.

The bark of *Quercus petraea* (Matt.) Liebl. (*Q. sessiliflora* Salisb, Fagaceae.) is official in both German and Swiss monographs. A decoction of *Quercus* cortex is used against unspecific diarrhea, inflammation of the oral, genital, and anal mucosa, and externally against inflammation of the skin (1). Tannins are regarded as the active compounds, and oligomeric proanthocyanidins have recently been isolated from the bark of *Q. petraea* (2). Castalagin and vescalagin (3) and insoluble ellagitannins (4) have been found in the wood of *Q. petraea*, but no information is available on the presence of hydrolyzable tannins in the bark. In this paper, the isolation and identification of four monomeric ellagitannins and six complex tannins from the bark of *Q. petraea* are reported. The antisecretory and the anthelmintic activity of the ellagitannin fraction as well as the astringency of several ellagitannins was examined.

RESULTS AND DISCUSSION

Partitioning of the 80% aqueous Me_2CO percolate of the freeze-dried bark of *Q. petraea* with Me_2CO, and fractionation of the aqueous layer by a combination of cc and droplet counter-current chromatography (dccc) afforded four monomeric ellagitannins, five flavanoellagitannins, and a procyanidinoellagitannin. 2,3-(*S*)-Hexahydroxydiphenoyl-glucose (HHDP-glucose) and pedunculagin were identified by direct comparison of their fabms and nmr data with authentic samples. Vescalagin and castalagin were identified by comparison of their fabms and nmr data with published results (5). In acutissimin A and eugenigrandin A [**1**], the ellagitannin moiety of vescalagin is connected through a C-C bond to (+)-catechin and to (+)-gallocatechin, respectively. Long-range heteronuclear correlation spectroscopy (HETCOR-LR) and ^{1}H-detected long-range heteronuclear multiple-bond correlation spectroscopy (HMBC) experiments with both complex tannins showed cross-peaks between H-2 and C-8a of the flavanol and between the polyol H-1 and C-7, C-8, and C-8a of the flavanol moiety. Thus, the flavanol is linked through C-8 to the C-1 of the polyol. HETCOR-LR and HMBC experiments with the regioisomeric compounds acutissimin B [**2**] and guajavin B [**3**], however, failed to show a cross-peak between H-2 and C-8a. This might be due to the preferred

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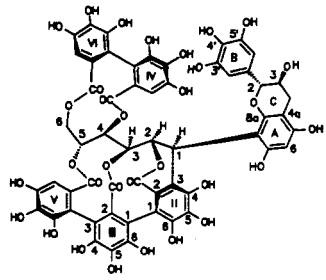

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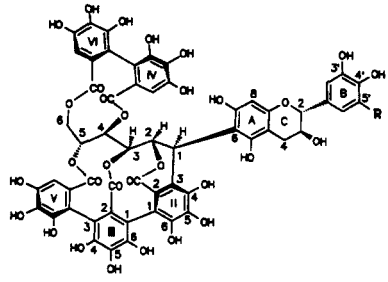

2 R=H
3 R=OH

equatorial orientation of the flavanol B-ring (*E*-conformation) of the compounds **2** and **3**. In rotating frame Overhauser enhancement spectroscopy (ROESY) nmr experiments, the spectrum of compound **3** showed a cross-peak between H-2 and H-4β, which resulted from the spacial vicinity of both protons in the *E*-conformers. The predominance of the *E*-conformation was corroborated by the $J_{2,3}$ coupling constant of 8.2 Hz, which suggests the pseudoaxial orientation of H-2 and H-3 (19). The identifications of acutissimin B [**2**] and guajavin B [**3**] were confirmed by comparison of their fabms, ^{1}H-nmr, and ^{13}C-nmr data with published results (7,8). The acutissimins A and B were previously isolated from the bark of several *Quercus* species and the bark of *Castanea crenata* Sieb. et Zucc. (7), and eugenigrandin A and guajavin B have been found in the bark of *Psidium guajava* L. (8). Recently, the stereochemistry of castalagin, vescalagin, and acutissimins A and B has been revised (9), and this was confirmed by our results from a ROESY experiment with guajavin B [**3**]. A distinct cross-peak between H-1 and H-3 of the polyol moiety resulting from the quasiplanar syn-orientation of both protons proved the rel-1*R* configuration. The nmr data of a further compound were indicative of a complex tannin with casuariin linked to (+)-catechin. All proton signals of both moieties were resolved in a ^{1}H-nmr spectrum recorded at 120°, and comparison of the ^{13}C-nmr and optical rotation data with published results identified this compound as stenophyllanin C, previously isolated from the bark of *Quercus stenophylla* Makino (10). The nmr spectra of another complex tannin revealed the signals of a vescalagin moiety and of a procyanidin. By fabms and optical rotation, this compound was identified as mongolicanin, previously isolated from *Quercus mongolica* Mizunara (11).

Flavanoellagitannins and procyanidinoellagitannins have been found in several Asian *Quercus* species (7, 10–12). This is the first report of complex tannins in a European *Quercus* species.

Mucosal application of 11–1100 μg/ml of the ellagitannin fraction showed a decrease of the prostaglandin E_2 (PGE_2) stimulated Cl^- secretion of 4–10%. This is a

rather weak effect, which might be due to the poor mucosal absorption of the ellagitannins, as has been shown for ellagic acid in mice (17) and for oligomeric proanthocyanidins from *Sorghum bicolor* (L.) Moench. in chickens (18). The observed antisecretory effect does not suffice to give a rationale to the use of the crude drug as an antidiarrheal. If preparations of oak bark are able to reduce diarrhea, other compounds or other effects must be involved (13).

The results of the determination of the astringency of extracts and of individual ellagitannins isolated from *Q. petraea* bark are presented in Table 1. The relative astringencies of the oak bark ellagitannins are rather low. This may be due to their rigid and inflexible structures which have been shown to substantially diminish the ability to complex with proteins (14). Though the ellagitannins represented 77% (by weight) of the total tannins in *Q. petraea* bark, they contributed only 45% to the astringency of the bark. Thus, the astringency of the crude drug is mainly due to its content of oligomeric proanthocyanidins (2).

TABLE 1. Relative Astringency of Extracts and of Ellagitannins from *Q. petraea* Bark.

	Mol wt	Relative Astringency[a]
Crude extract[b]	—	0.40
Ellagitannin fraction	—	0.43
Proanthocyanidin fraction	—	1.14
Pedunculagin	784	0.20
Vescalagin	934	0.24
Stenophyllanin C	1056	0.25
Acutissimin A	1206	0.28
Eugenigrandin A [**1**]	1222	0.46
Guajavin B [**3**]	1222	0.44

[a]Relative to tannin (Roth) (1.00).
[b]Percolate with 80% Me_2CO.

In an in vitro anthelmintic assay with twenty medicinal plants used against gastrointestinal disorders, the aqueous extract of *Q. petraea* bark showed interesting activity; thus, we tested the tannin fractions separately. The fraction containing ellagitannins and complex tannins inhibited the reproduction of the soil nematode *Caenorabditis elegans* with an IC_{50} of 500 ppm. Motility of the nematodes was only slightly affected. Under the same conditions, the synthetic anthelmintic mebendazole had an IC_{50} of 10 ppm. The proanthocyanidins of *Q. petraea* bark had an IC_{50} of 125 ppm in this assay. Although the oak tannins show some nematodicidal activity, this effect is too weak to support the use of *Q. petraea* bark as an anthelmintic.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Fabms: negative-ion mode, solvent, H_2O; matrix, glycerol or glycerol-thioglycerol; bombardment, Cs, 8 keV or Xe, 7 keV. Nmr, 400 and 600 MHz (^1H), 100 and 150 MHz (^{13}C); HETCOR experiments, delays adjusted to 0.05 sec (10 Hz) and 0.1 sec (5 Hz) for $^2J_{CH}$ and $^3J_{CH}$ couplings and to 0.0036 sec (150 Hz) for $^1J_{CH}$ couplings; HMBC experiments, 600/150 MHz, spectra recorded at 80° and 120°. Sephadex LH-20 (Pharmacia), MCI gel CHP-20P, 37–75 μm (Mitsubishi Chemical Industries) and Fractogel TSK HW-40(S) (Merck) were used for cc. Dccc (275 glass columns, 2.0×400 mm) was carried out with 1-BuOH-1-PrOH-H_2O (2:1:3, ascending mode). Tlc, Si gel 60 F_{254} (Merck); EtOAc-HCOOH-H_2O (90:5:5). 2D-tlc, Cellulose F (Merck); HOAc 6% (A), 2-BuOH-HOAc-H_2O (14:1:5) (B), visualization reagent: vanillin-EtOH/H_2O 5%-concentrated HCl (0.5:75:25).

PLANT MATERIAL.—The bark of the younger branches of an approximately 30-year old *Q. petraea* tree was collected near Freiburg/B. at 600 m altitude in April 1989, freeze-dried, and powdered. A voucher

specimen (No. 2105) was deposited at the herbarium of the Institut für Pharmazeutische Biologie at Freiburg.

EXTRACTION AND ISOLATION.—The powdered bark of *Q. petraea* was extracted with Me_2CO-H_2O (8:2), the Me_2CO removed *in vacuo*, with the aqueous layer washed with petroleum ether, and then extracted with EtOAc (2). A 280-g aliquot of the lyophilized H_2O layer (493 g) was chromatographed on Sephadex LH-20 (55×600 mm) with EtOH-H_2O (1:1, 34 liters) to give five fractions F1-F5. A part (4 g) of F1 (13.1 g, 6.5–11.2 liters) was further separated on MCI gel (35×500 mm) with MeOH-H_2O (25:75) followed by dccc and by cc over Sephadex LH-20 (20×400 mm) with EtOH-H_2O (1:1) to give 2,3-(*S*)-HHDP-glucose (120 mg, 0.24–0.345 liters). F2 (4.1 g, 11.2–15.8 liters) was separated over MCI gel (25×450 mm) with H_2O (2 liters) followed by MeOH-H_2O (1:9) to give vescalagin (100 mg, 0.24–0.41 liters). F3 (18.8 g, 15.8–18.3 liters) was chromatographed over MCI gel (45×600 ml) with H_2O (4 liters) followed by MeOH-H_2O (1:9) to yield castalagin (1500 mg, 1.0–1.5 liters). F4 (24.3 g, 18.3–31.1 liters) was separated over MCI gel (45×600 mm) with H_2O (4.5 liters) followed by MeOH-H_2O (15:85, 10 liters) to give fraction 4.1 (4.9 g, 2.6–8.2 liters) and fraction 4.2 (1.9 g, 14–14.5 liters). Fraction 4.1 contained three phenolic compounds which were separated by cc on MCI gel (25×450 mm) with MeOH-H_2O (15:85) yielding a fraction (1.38–3.36 liters) which was purified by dccc to give eugenigrandin A [**1**] (280 mg, 0.1–0.12 liters) and stenophyllanin C (503 mg, 0.135–0.17 liters), and a further fraction (3.36–4.56 liters) which was purified by cc on Fractogel TSK (19×450 mm) with EtOH-H_2O-Me_2CO (6:3:1) to yield acutissimin A (1400 mg, 0.65–0.75 liters). Fraction 4.2 was chromatographed over MCI gel with H_2O (1.0 liters) and MeOH-H_2O (5:95–25:75) in steps of 5% (1.5 liters each). The 25% MeOH eluate was finally purified by dccc (descending mode) to give acutissimin B [**2**] (150 mg, 0.036–0.072 liters). Ascending dccc of the MeOH-H_2O 10% eluate yielded mongolicanin (70 mg, 0.09–0.12 liters). Fraction 5 (23.8 g, 31.1–34.0 liters) was rechromatographed over MCI gel (45×600 mm) with MeOH-H_2O (15:85, 0.3 liters) followed by ascending dccc to give pedunculagin (273 mg). The stationary dccc phase was purified on Fractogel as described above to give guajavin B [**3**] (50 mg, 0.2–0.3 liters).

Acutissimin A.—White amorphous residue: fabms, ^{1}H-nmr, and ^{13}C-nmr data in agreement with literature values (7).

Eugenigrandin A [**1**].—White amorphous residue: $[\alpha]^{31}D$ −15.5° (*c*=0.8, Me_2CO); fabms *m/z* $[M-H]^-$ 1221.4. ^{1}H nmr (600 MHz, Me_2CO-d_6, standard, Me_2CO-d_5 2.0566), δ flavanol: 2.42 (1H, d, *J*=15 Hz, H-4), 2.86 (1H, d, *J*=15 Hz, H-4), 4.48 (1H, br s, H-3), 5.42 (1H, s, H-2), 6.13 (1H, s, H-6), 6.51 (2H, s, H-2′, H-6′); polyol: 4.10 (1H, d, *J*=12 Hz, H-6), 4.63 (1H, d, *J*=12 Hz, H-6), 4.73 (1H, d, *J*=7.5 Hz, H-3), 4.80 (1H, s, H-1), 5.15 (1H, s, H-2), 5.25 (1H, t, *J*=7.5 Hz, H-4), 5.60 (1H, d, *J*=7.5 Hz, H-5); nonahydroxytriphenoyl: 6.75 (1H, s, H-3, ring V); hexahydroxydiphenoyl: 7.04 (1H, s, H-3, ring IV), 6.59 (1H, s, H-3, ring VI). ^{13}C nmr (150 MHz, Me_2CO-d_6, standard, Me_2CO-d_5 28.6340), δ flavanol: 23.43 (C-4), 67.70 (C-3), 80.09 (C-2), 96.88 (C-6), 97.73 (C-4a), 105.04 (C-8), 105.32 (2C, C-2′, C-6′), 131.22 (C-1′), 132.95 (C-4′), 146.21 (2C, C-3′, C-5′), 152.83 (C-8a), 155.82 (C-5), 157.29 (C-7); polyol: 37.55 (C-1), 65.45 (C-6), 71.00 (C-4), 71.27 (C-5), 72.25 (C-3), 77.40 (C-2); nonahydroxytriphenoyl, ring II: 116.63[a] (C-1), 120.25 (C-3), 127.58 (C-2), 135.17[b] (C-5), 143.19 (C-4), 143.54[c] (C-6), 167.53 (carbonyl C); ring III: 113.18[a] (C-1), 113.44 (C-3), 124.91[d] (C-2), 136.50[b] (C-5), 144.14 (C-4), 144.59[c] (C-6), 165.61 (carbonyl C); ring V: 108.93 (C-3), 114.23 (C-1), 125.54 (C-2), 136.37 (C-5), 144.69[c] (C-6), 145.50 (C-4), 167.40 (carbonyl C); hexahydroxydiphenoyl, ring IV: 107.11 (C-3), 115.87 (C-1), 126.92[d] (C-2), 136.69 (C-5), 144.83[c] (C-6), 145.07 (C-4), 167.17 (carbonyl C); ring VI: 106.90 (C-3), 115.02 (C-1), 128.10[d] (C-2), 135.61 (C-5), 145.33[c] (2C, C-4, C-6), 169.09 (carbonyl C). Chemical shifts with the same superscript are interchangeable.

Acutissimin B [**2**].—Off-white amorphous residue: fabms, ^{1}H-nmr, and ^{13}C-nmr data in agreement with literature data (7).

Guajavin B [**3**].—White amorphous residue: $[\alpha]^{31}D$ −67.9° (*c*=0.8, MeOH); the optical rotation differed significantly from the published value $[\alpha]^{21}D$ +27.5° (*c*=1.1, MeOH) (8), but fabms, ^{1}H-nmr, and ^{13}C-nmr data were in agreement with literature data (8).

Stenophyllanin C.—White amorphous residue: fabms *m/z* $[M+Na]^+$ 1079.4, *m/z* $[M+K]^+$ 1095.3. Optical rotation, fabms, ^{1}H-nmr, and ^{13}C-nmr data in agreement with literature data (10).

Mongolicanin.—White amorphous residue: optical rotation, fabms, ^{1}H-nmr, and ^{13}C-nmr data in agreement with literature data (11).

Castalagin.—Colorless rhomboid crystals; mp 220° (dec). Optical rotation, fabms, ^{1}H-nmr, and ^{13}C-nmr data in agreement with published results (5).

Vescalagin.—White amorphous residue: optical rotation, fabms, ^{1}H-nmr, and ^{13}C-nmr data in agreement with published results (5).

BIOASSAYS.—The relative astringencies were determined by hemanalysis with fresh human blood (15). The anthelmintic activity was tested with the soil nematode *Caenorhabditis elegans* as previously described (2). The antisecretory activity was determined using the isolated rabbit colon mounted in an Ussing chamber as a model (16). Cl^- secretion was induced with 1 μmol/l prostaglandin E_2 (serosal) after mucosal application of 0.1 mmol/liter amiloride. The ellagitannin fraction was tested in concentrations of 11 mg/liter, 110 mg/liter, and 1100 mg/liter.

ACKNOWLEDGMENTS

We thank Dr. V. Wray (G.B.F., Braunschweig) for HMBC nmr spectra, Prof. Dr. H.H. Linde, Pharmazeutisches Institut, Universität Basel, and Dr. R.G. Graf, Institut für Organische Chemie, Universität Saarbrücken, for fabms spectra. Mrs. I. Burhoff, Physiologisches Institut, Universität Freiburg, kindly performed the antisecretory assays.

LITERATURE CITED

1. Aufbereitungsmonographie der Kommission E am BGA "Quercus Cortex," Banz Nr. 22a, December 14, 1989.
2. E. Pallenbach, E. Scholz, M. König, and H. Rimpler, *Planta Med.*, **59**, 262 (1993).
3. W. Mayer, H. Seitz, J.C. Jochims, K. Schauerte, and G. Schilling, *Liebigs Ann. Chem.*, **751**, 60 (1971).
4. S. Peng, A. Scalbert, and B. Monties, *Phytochemistry*, **30**, 775 (1991).
5. Y. Itakura, G. Habermehl, and D. Mebs, *Toxicon*, **25**, 1291 (1987).
6. M. König, "Ellagitannine aus *Quercus petraea* (Matt.) Liebl." Ph.D. Thesis, Albert-Ludwigs-Universität, Freiburg, Germany (1991).
7. K. Ishimaru, G.I. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 602 (1987).
8. T. Tanaka, N. Ishida, M. Ishimatsu, G.I. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **40**, 2092 (1992).
9. G.I. Nonaka, T. Sakai, T. Tanaka, K. Mihashi, and I. Nishioka, *Chem. Pharm. Bull.*, **38**, 2151 (1990).
10. G.I. Nonaka, H. Nishimura, and I. Nishioka, *J. Chem. Soc., Perkin Trans. I*, 163 (1985).
11. K. Ishimaru, M. Ishimatsu, G.I. Nonaka, K. Mihashi, Y. Iwase, and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 3319 (1988).
12. G.I. Nonaka, K. Ishimaru, K. Mihashi, Y. Iwase, M. Ageta, and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 857 (1988).
13. C. Geiger, E. Scholz, and H. Rimpler, *Planta Med.*, **60**, 384 (1994).
14. E. Haslam, *Nat. Prod. Rep.*, **3**, 217–249 (1986).
15. E.C. Bate-Smith, *Phytochemistry*, **12**, 907 (1973).
16. R. Greger, R.B. Nitschke, E. Lohrmann, I. Burhoff, M. Hropot, H.C. Englert, and H.J. Lang, *Pflügers Arch.*, **419**, 190 (1991).
17. R.W. Teel and R.M. Martin, *Xenobiotica*, **18**, 397 (1988).
18. L.M. Jimenez-Ramsey, J.C. Rogler, T.L. Housley, L.G. Butler, and R.G. Elkin, *J. Agric. Food Chem.*, **42**, 963 (1994).
19. L.J. Porter, R.Y. Wong, M. Benson, B.G. Chan, V.N. Vishwanadhan, R.D. Gandour, and W.L. Mattice, *J. Chem. Res. (S)*, 86 (1986).

Received 23 March 1994