LIGNAN GLYCOSIDES FROM THE HEARTWOOD OF EUROPEAN OAK QUERCUS PETRAEA

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ABSTRACT.—(+)-Lyoniresinol [1] and (−)-lyoniresinol [ent-1] (the former being more abundant), (+)-lyoniresinol 2a-O-β-D-glucopyranoside [3], lyoniside [4], and a new metabolite, namely (−)-lyoniresinol 2a-O-β-D-xylopyranoside [ent-4][1], have been isolated from *Quercus petraea* heartwood. Their structures were deduced from chemical and spectral data.

It is well known that wood extractives are involved in the modification of flavor during the aging of whisky and brandy in oak casks (1). Thus, a knowledge of the chemical composition of the wood used for constructing such casks is essential to a study of aging and relevant from a phytochemical point of view. We report here a study on the heartwood of *Quercus petraea* Liebl. (= *Quercus robur* L. ssp. *sessiflora* D.C. = *Quercus sessilis* Ehur.) (Fagaceae), which led to the isolation and structural determination of five disyringyl lignans having an aryl-tetralin skeleton, namely (+)- and (−)-lyoniresinol (the former, **1**, prevailing in the mixture) (2–6), (+)-lyoniresinol 2a-O-β-D-glucoside [**3**] (7), lyoniside [**4**] (5, 8–11), and (−)-lyoniresinol 2a-O-β-D-xyloside [ent-**4**][1]. The last compound had not been reported so far.
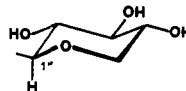
Chips of *Q. petraea* heartwood were extracted with aqueous Me_2CO, and the extract, after removal of the Me_2CO, was partitioned between H_2O and EtOAc. The product obtained from the EtOAc solution was found to be lyoniresinol [represented as the (+) form in formula **1**] on the basis of its spectral properties (2,4,6,7) and by comparison,
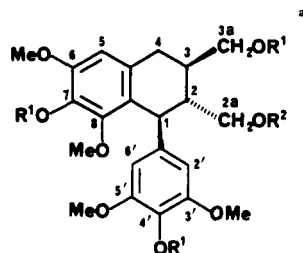
1 $R^1=R^2=H$
2 $R^1=R^2=Ac$
3 $R^1=H, R^2=$

4 $R^1=H, R^2=$

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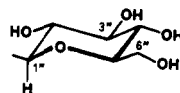
[a]Our numbering of the aryl-tetralin skeleton is according to Hulbert *et al.* (12).

after acetylation, with an authentic sample of (+)-lyoniresinol tetraacetate [**2**] (2,6). When examined for its optical purity, our sample of lyoniresinol showed a value of $[\alpha]^{20}D$, much lower than that reported for the (+) form (7). Such value, as well as the cd spectrum, did not change significantly after repeated purification (hplc), thus indicating the absence of optically active impurities. That the isolated product was a

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[1]The term *ent* refers to the aglycone moiety.

nonracemic mixture of lyoniresinol was confirmed by the peak splitting observed in the ^{1}H-nmr spectrum of its tetraacetate after addition of Eu(fod)$_3$. Single peak integration of the nonracemic lyoniresinol spectrum allowed the excess of the (+) form **1** to be estimated at 20%. This value was in good agreement with that calculated from the optical rotation and the cd spectrum assuming $[\alpha]^{20}$D 58.0° (MeOH, $c = 0.5$) (7) and $\Delta\epsilon$ + 10.8 (at λ 244 nm in MeOH) (12) for the pure enantiomer.

The first compound isolated from the aqueous extract (see Experimental) appeared to be (+)-lyoniresinol 2a-O-β-D-glucopyranoside [**3**] on the basis of its acid hydrolysis, giving rise to enantiomerically pure (+)-lyoniresinol [**1**] and D-glucose. Structure **3** was confirmed by comparison of its ^{1}H- and ^{13}C-nmr spectra with those reported by Miyamura *et al.* (7).

Further fractionation of the aqueous extract by Sephadex chromatography afforded a mixture of two compounds indistinguishable in tlc (ratio 1:1 in reversed-phase hplc and by the peak splitting in ^{1}H- and ^{13}C-nmr spectra). A preliminary hydrolysis of this mixture gave racemic lyoniresinol and D-xylose as the only reaction products, thus indicating an isomeric (probably diastereomeric) relationship between the two compounds. When obtained in very pure form by preparative hplc, the faster moving compound was spectroscopically identical to lyoniside [**4**] (4,8) (Table 1), and, as expected, it afforded (+)-lyoniresinol by acid hydrolysis. By the same treatment, the slower moving compound gave rise to (−)-lyoniresinol. Furthermore, inspection of its ^{13}C-nmr spectrum (Table 1) revealed a strict correspondence in the signals with lyoniside [**4**]. These data allowed the structure of (−)-lyoniresinol 2a-O-β-D-xylopyranoside [*ent*-**4**] to be assigned to the compound accompanying lyoniside [**4**]. The β-glycosidic bond in this compound, as well as in the other isolated

TABLE 1. ^{13}C-nmr Chemical Shifts (pyridine-d_5, ppm) of **4** and *ent*-**4**.

Carbon	**4**[a]	*ent*-**4**
C-1	42.7	42.8
C-2	46.3	46.3
C-3	40.9	40.8
C-4	33.9	34.1
C-4a	129.6	129.6
C-5	108.9	108.9
C-6	148.1[b]	148.2[b]
C-7	138.8	138.8
C-8	148.4[b]	148.4[b]
C-8a	126.6	126.7
C-2a	71.1	70.6
C-3a	65.7	65.5
C-1'	139.6	139.6
C-2'	107.6	107.5
C-3'	149.3	149.3
C-4'	135.7	135.7
C-5'	149.3	149.3
C-6'	107.6	107.5
3',5'-OMe	56.6	56.5
6-OMe	56.2	56.2
8-OMe	59.8	59.6
C-1″	105.6	105.8
C-2″	75.0	75.0
C-3″	78.7	78.7
C-4″	71.3	71.3
C-5″	67.5	67.4

[a]Measured in DMSO-d_6 by Vecchietti *et al.* (4).
[b]Assignments may be reversed.

lyoniresinol glycosides, was proven by the value of the coupling constant ($J = 8$ Hz) between the anomeric proton and that in C-2″ position.

To our knowledge, no glycoside of (−)-lyoniresinol has been reported so far, the only known natural occurrence of this enantiomer being as an aglycone in racemic mixture (2–6).

EXPERIMENTAL

spectra were obtained on a VG 707EQ mass spectrometer. Microanalyses were obtained with a Perkin-Elmer 240 elemental analyser. Cd spectra were recorded on a Jasco J-500 instrument. Tlc was performed using precoated Si gel plates (Merck, 0.25 mm layer) and the following eluents: A, $CHCl_3$-EtOAc-EtOH (5:4:1); B, *n*-BuOH-HOAc-H_2O (4:1:2); C, HOAc-$CHCl_3$-H_2O (35:30:5); spots were visualized by spraying with 50% H_2SO_4. Commercial Merck Si gel 60 (230–400 mesh ASTM) was used for flash chromatography with $CHCl_3$-EtOAc-EtOH (5:4:1) as eluent. Medium pressure liquid chromatography was performed on a Jobin Yvon system using ICN Biomedicals Si gel (32–63 mesh ASTM) as stationary phase and eluent as above. Hplc was performed on a Perkin-Elmer Series 3B liquid chromatograph connected to a variable wavelength uv detector (Perkin-Elmer LC-75 Spectrophotometric detector). Analytical conditions were: column 250×4 mm, LiChrosorb RP-18, 10 μ; flow rate 1 ml/min; detector λ 280 nm; eluent MeOH/H_2O, linear gradient from 20 to 70% MeOH in 30 min. Semipreparative conditions were: column 250×25 mm, LiChrosorb RP-18, 7 μ; flow rate 15 ml/min; detector λ 280 nm; eluent MeOH/H_2O, linear gradient from 20 to 70% MeOH in 30 min. The Sephadex LH-20 (particle size 25–100 μ) was supplied by Pharmacia Fine Chemicals AB.

PLANT MATERIAL.—Heartwood of Yugoslavian oak *Q. petraea* was used in this study. A voucher specimen is deposited at Centro Studi Maria Branca, Milan.

EXTRACTION.—The dried heartwood of *Q. petraea* (8 kg) was extracted with 80% aqueous Me_2CO (50 liters) at room temperature for 48 h. After concentration under reduced pressure and filtration of insoluble material, the aqueous solution was extracted with EtOAc (10×500 ml). The combined EtOAc extracts were concentrated in vacuo to afford a pale brown solid (50 g, residue I). On lyophilization of the aqueous solution (lower layer), 350 g of a brown mass was obtained (residue II).

ISOLATION OF (+)-LYONIRESINOL [1].—Residue I (800 mg) was subjected to flash chromatography monitoring by tlc (eluent A). Fractions containing lyoniresinol as a major component (R_f 0.30) were combined. Further purification by medium pressure liquid chromatography and final isolation by semi-preparative hplc afforded 1 (80 mg), as an amorphous white solid, which was found to be pure both on tlc (eluent A) and analytical hplc (Rt 19 min) (0.01 % from the starting material): mp 176–179°; $[\alpha]^{20}D$ +13.3° (c = 0.32, MeOH); cd (c = 2.38×10^{-4}, MeOH) $[\theta]^{25}$ (nm) −396 (285), +1650 (274), +6732

(244); uv λ max (MeOH) 278 (log ε 3.65), 280 (3.65); ^1H nmr (pyridine-d_5) δ 6.93 (2H, s, H-2', H-6'), 6.78 (1H, s, H-5), 5.11 (1H, d, J = 5.5 Hz, H-1), 4.24 (1H, dd, J_{3-3a} = 5.0 Hz, J_{gem} = 10.5 Hz, H-3a), 4.18 (2H, d, J_{2-2a} = 5.0 Hz, H-2a), 4.16 (1H, dd, J_{3-3a} = 5.0 Hz, J_{gem} = 10.5 Hz, H-3a), 3.85 (3H, s, OMe-8), 3.84 (3H, s, OMe-6), 3.73 (6H, s, OMe-3' and OMe-5'), 3.19 (2H, br d, J = 5.0 Hz, H-4), 2.75 (1H, m, H-2), 2.33 (1H, m, H-3); ^{13}C nmr see Vecchietti *et al.* (4) and Miyamura *et al.* (7); eims *m/z* 420 (100%), 402 (6), 389 (5), 371 (21), 248 (12), 217 (33), 210 (15), 205 (43), 183 (54), 167 (48). Calcd for $C_{22}H_{28}O_8$, C 60.27, H 6.86; found C 59.95, H 7.15%.

LYONIRESINOL TETRAACETATE [2].—Compound 1 (50 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (10 ml) (24 h at room temperature). Usual workup and crystallization from MeOH gave 2 (48 mg), which was identified as lyoniresinol tetraacetate by tlc (eluent A) and nmr-data comparison with an authentic sample (racemic).

ISOLATION OF (+)-LYONIRESINOL 2a-O-β-D-GLUCOPYRANOSIDE [3].—Residue II (100 g, in three portions) was chromatographed on Sephadex LH-20 (300 g) using MeOH-H_2O (3:7) as eluent. The eluate was collected in 10-ml fractions which were examined by tlc (eluent B). Fractions 2 and 3, when combined and purified by semipreparative hplc, gave pure 3 (35 mg), as an amorphous white powder: mp 178–180°; $[\alpha]^{20}D$ +23.2 (c = 0.504, MeOH) [lit. (7) $[\alpha]^{20}D$ +22.4 (c = 1.01, MeOH)]; cd (c = 1.5×10^{-4}, MeOH) $[\theta]^{25}$ (nm) −924 (284), +5676 (268), +10923 (244); uv λ max (MeOH) 276 (log ε 3.68); ^1H nmr (pyridine-d_5) δ 7.06 (2H, s, H-2', H-6'), 6.74 (1H, s, H-5), 5.17 (1H, d, J = 5.5 Hz, H-1), 4.25 (1H, d, J = 7.5 Hz, H-1''), 3.77 and 3.75 (6H, 2s, OMe-8 and OMe-6), 3.70 (6H, s, OMe-3' and OMe-5'), 3.18 (1H, dd, J_{gem} = 14.4 Hz, J_{4-3} = 11.7 Hz, H-4$_{ax}$), 3.08 (1H, dd, J_{gem} = 14.4 Hz, J_{4-3} = 4.0 Hz, H-4$_{eq}$), 2.73 (1H, m, H-2), 2.14 (1H, m, H-3), the remaining protons gave complex overlapping signals in the region 3.0–4.5 δ; ^{13}C nmr see Miyamura *et al.* (7); fabms *m/z* $[M + Na]^+$ 605, $[M]^+$ 582, $[aglycone]^+$ 420. Calcd for $C_{28}H_{38}O_{13} \cdot H_2O$, C 55.99, H 6.68%; found C 55.55, H 6.90%.

A solution of 3 (20 mg) was refluxed in 0.5 N H_2SO_4 (10 ml) for 18 h at 80° and extracted with EtOAc. Evaporation of this extract and purification of the residue by semipreparative hplc yielded the aglycone which exhibited the ^1H-nmr data, optical rotation, and cd spectrum of (+)-lyoniresinol [1] (7). D-Glucose in the aqueous layer was identified by tlc comparison with an authentic sample (eluent C, detection by aniline-diphenylamine reagent) (13). (+)-Lyoniresinol

obtained from acid hydrolysis of **3** was acetylated as above. The resulting (+)-lyoniresinol tetraacetate was shown to be enantiomerically pure by ^{1}H-nmr reagent shift experiments [Eu(fod)$_3$].

ISOLATION OF LYONISIDE [**4**] AND (−)-LYONIRESINOL   2a-*O*-β-D-XYLOPYRANOSIDE [*ent*-**4**].—Fractions 4–6 arising from the Sephadex chromatography of residue II, when examined by analytical hplc, were found to contain only two compounds (ratio *ca.* 1:1) with very close Rt's. Part of the product obtained from evaporation of these fractions (40 mg) was refluxed in 0.5 N H$_2$SO$_4$ (10 ml) for 18 h at 80°. After cooling, the reaction mixture was extracted with EtOAc. The organic layer was dried, evaporated in vacuo, and purified by semipreparative hplc to give a solid (15 mg) that was acetylated in the usual manner. The reaction product was shown to be identical in all respects [^{1}H nmr, ^{13}C nmr, tlc (eluent A)] with racemic lyoniresinol tetraacetate [**2**]. The presence of D-xylose in the aqueous layer was proved by tlc analysis (as described above for D-glucose) and by the sign of its optical rotation.

The remainder of the product partially used for the previous hydrolysis (60 mg) was subjected to semipreparative hplc to afford two compounds (I and II) pure in analytical hplc. Both appeared as amorphous powder.

Compound I (lyoniside [**4**]) (18 mg): R_f 0.58; Rt 17.22 min; mp 162–164° [lit. (10) mp 164–165°]; [α]^{20}D +27.3° (*c* = 1.71, MeOH) [lit. (10) [α]^{20}D +26.7°]; uv λ max (MeOH) 276–278 (log ε 3.74); ^{1}H nmr (pyridine-*d$_5$*) δ 7.01 (2H, s, H-2′, H-6′), 6.70 (1H, s, H-5), 5.07 (1H, d, *J* = 6.0 Hz, H-1), 4.78 (1H, d, *J* = 8.0 Hz, H-1″), 3.62 (3H, s, OMe-6), 3.57 (6H, s, OMe-3′, OMe-5′), 3.53 (3H, s, OMe-8), 3.13 (1H, dd, *J$_{gem}$* = 14.3 Hz, *J$_{4-3}$* = 11.5 Hz, H-4$_{ax}$), 3.01 (1H, dd, *J$_{gem}$* = 14.3 Hz, *J$_{4-3}$* = 4.0 Hz, H-4$_{eq}$), 2.64 (1H, m, H-2), 2.10 (1H, m, H-3), the remaining protons gave complex overlapping signals in the region 3.0–4.5 δ; ^{13}C nmr see Table 1; fabms *m/z* [M + Na]$^+$ 575, [M]$^+$ 552, [aglycone]$^+$ 420. Calcd for C$_{27}$H$_{36}$O$_{12}$·H$_2$O, C 56.47, H 6.87%; found C 56.83, H 6.68%.

Compound II [*ent*-**4**] (22 mg): R_f 0.58; Rt 17.73 min; mp 157–160°; [α]^{20}D −63.9 (*c* = 0.143, MeOH); uv λ max (MeOH) 277 (log ε 3.6); ^{1}H nmr (pyridine-*d$_5$*) δ 6.92 (2H, s, H-2′, H-6′), 6.69 (1H, s, H-5), 4.96 (1H, d, *J* = 6 Hz, H-1), 4.69 (1H, d, *J* = 8.0 Hz, H-1″), 3.63 (3H, s, OMe-6), 3.58 (6H, s, OMe-3′, OMe-5′),

3.51 (3H, s, OMe-8), 3.13 (1H, dd, *J$_{gem}$* = 14.3 Hz, *J$_{4-3}$* = 11.5 Hz, H-4$_{ax}$), 2.96 (1H, dd, *J$_{gem}$* = 14.3 Hz, *J$_{4-3}$* = 4.0 Hz, H-4$_{eq}$), 2.64 (1H, m, H-2), 2.10 (1H, m, H-3), the remaining protons gave complex overlapping signals in the region 3.0–4.5 δ; ^{13}C nmr see Table 1; fabms *m/z* [M + Na]$^+$ 575, [M]$^+$ 552, [aglycone]$^+$ 420. Calcd for C$_{27}$H$_{36}$O$_{12}$·H$_2$O, C 56.47, H 6.87%; found C 56.03, H 7.01%.

LITERATURE CITED

1. J.S. Swan, in: "Handbook of Food and Beverage Stability." Ed. by G. Charalambous, Academic Press, New York, 1986, pp. 801–833.
2. K. Nabeta, J. Yonekubo, and M. Miyake, *Mokuzai Gakkaishi*, **33**, 408 (1987); *Chem. Abstr.*, **107**, 117138 (1987).
3. M. Varma, R.S. Varma, and M.R. Parthasarathy, *Z. Naturforsch. C: Biosci.*, **35C**, 344 (1980).
4. V. Vecchietti, G. Ferrari, F. Orsini, and F. Pelizzoni, *Phytochemistry*, **18**, 1847 (1979).
5. M.K. Seikel, F.D. Hostettler, and G.J. Niemann, *Phytochemistry*, **10**, 2249 (1971).
6. F.D. Hostettler and M.K. Seikel, *Tetrahedron*, **25**, 2325 (1969).
7. M. Miyamura, T. Nohara, T. Tomimatsu, and I. Nishioka, *Phytochemistry*, **22**, 215 (1983).
8. J. Sakakibara, I. Hiroji, and Y. Masaiti, *Yakugaku Zasshi*, **94**, 1377 (1974); *Chem. Abstr.*, **82**, 73392g (1975).
9. N. Narasimhachari and E. von Rudloff, *Phytochemistry*, **12**, 2551 (1973).
10. N. Narasimhachari and E. von Rudloff, *Can. J. Chem.*, **40**, 1118 (1962).
11. Y. Kato, N. Kato, and M. Baba, *Pharmazie*, **39**, 425 (1984).
12. P.B. Hulbert, W. Klyne, and P.M. Scopes, *J. Chem. Res. Synop.*, 27 (1981).
13. B.A. Lewis and F. Smith, in: "Thin-Layer Chromatography." Ed. by E. Stahl, Springer-Verlag, Berlin, 1969, p. 811.