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Ralf Lutterbach, Joachim Stöckigt, and Heinz Kolshorn

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p-HYDROXYPHENYL-*O*-β-D-PRIMEVEROSIDE, A NOVEL GLYCOSIDE FORMED FROM HYDROQUINONE BY CELL SUSPENSION CULTURES OF *RAUWOLFIA SERPENTINA*[1]

RALF LUTTERBACH, JOACHIM STÖCKIGT,*

Institute of Pharmacy, Department of Pharmaceutical Biology; University of Mainz, Staudinger Weg 5, 55099 Mainz, Germany

and HEINZ KOLSHORN

Institute of Organic Chemistry, University of Mainz, Becherweg 18-22, 6500 Mainz, Germany

ABSTRACT.—From cell suspension cultures of *Rauwolfia serpentina* which were continuously treated with hydroquinone for 7 days, a novel glycoside **1** was formed in yields up to 5.8 g/liter of working volume. Isolation and structure elucidation showed this compound to be the *O*-β-D-primeveroside of hydroquinone.

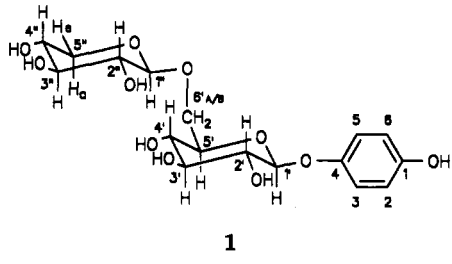
Our recent investigation of the biotransformation of hydroquinone by cell suspension cultures of *Rauwolfia serpentina* Benth. (Apocynaceae) resulted in the high production of *p*-hydroxyphenyl-*O*-β-D-glucoside (arbutin) ranging up to 18 g/liter of working volume (1). This is the highest transformation rate ever observed with a plant cell culture system for a single natural product. The hplc analysis of the cell and medium extracts indicated the formation of a further glycoside of hydroquinone as a side product found in about 5 g/liter. Here we report the isolation and structure determination of this glycoside, which has not yet been reported as a natural product; it is the primeveroside of hydroquinone.

When the nutrition medium of a feeding experiment of *Rauwolfia* cells with high amounts of hydroquinone (HQ) was analyzed by hplc, a further product at Rt 6.8 min was detected besides arbutin (1). This compound showed on tlc a significantly higher polarity than arbutin but gave the same blue color after spraying with $FeCl_3/K_3[Fe(CN)_6]$ solution. These properties of the unknown product pointed to a glycosidic structure, most probably to a diglycoside of HQ. From earlier experiments with enzymes of wheat germ it was suggested that in addition to arbutin an appropriate diglucoside was formed (2). However, during recent work on the biotransformation of HQ with cell suspension cultures of *Catharanthus roseus* G. Don, higher glycosides of HQ like the diglucoside could not be observed (3).

Therefore, a rigorous proof of the structure of the compound was necessary. Its uv data nearly coincided with those of arbutin indicating the same chromophore. The fabms measurements showing the molecular ion $[M-H]^-$ at *m/z* 403.5 disproved the structure of a *p*-hydroxyphenyl-diglucoside. The mol wt, however, suggested an appropriate derivative omitting a CH_2OH group and favoring a diglycoside with a pentose and hexose unit. Because the ^1H nmr exhibited a free phenolic OH at 9.05 ppm, the sugar moieties must be connected to each other. The ^1H-nmr data measured in DMSO-d_6/pyridine-d_5/D_2O revealed glycosidic protons at C-1′ and C-1″ at 4.80 and 4.36 ppm. This assignment was also consistent with the ^{13}C data for both carbons (C-1″ at 104.33 ppm and C-1′ at 102.24 ppm). These data were in agreement with the two β-glycosidic linkages in the molecule where the *p*-hydroxyphenyl residue is connected to the 1′ β-OH group of the glucose unit. The remaining pentose

[1]Dedicated to the memory of Professor Edward Leete.

unit is then bound to the 6′-OH group. In fact, double resonance and COSY experiments showed all of the connections in the glucose moiety between H-1′ and H-5′ with transdiaxial coupling constants as well as the coupling of H-5′ to both protons of C-6′. Starting with the double resonance experiments at H-1″ of the pentose part, large coupling constants (J=ca. 7–9 Hz) were found between H-1″, -2″, -3″, -4″ and the axial H-5″. Exclusively, one small coupling (J=5.3 Hz) was detected between H-4″ and the equatorial H-5″. All these measurements were indicative for a xylose moiety to be the second sugar in the molecule. NOe measurements between H-1″, H-3″, and H_a-5″ revealed significant effects which, in addition to the above-mentioned data, clearly ruled out ribose being part of the novel glycoside and, finally, confirmed the structure of *p*-hydroxyphenyl-*O*-β-D-primeveroside [*p*-hydroxyphenyl-*O*-β-D-xylopyranosyl-(1″↦6′)-β-D-*gluco*-pyranoside] [**1**].



1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Cultivation of *R. serpentina* cell suspensions and the treatment with hydroquinone were performed as previously described (1). Hplc analyses were carried out on a Merck-Hitachi System (L-6200 A Pump, L-4250 UV-Vis Detector, AS-2000 Autosampler, D-2500 Chromato-Integrator) in combination with a LiChroCart^R 125-4 Superspher 100 column. The solvent system, applied with a flow rate of 1 ml/min, was MeOH (5%) pH 2.5 adjusted with H_3PO_4 (detection at 230 nm); Rt of **1** was 6.8 min. *p*-Hydroxyphenyl-primeveroside [**1**] was isolated by chromatography using 0.5 mm Si gel plates G/uv_{254} (Merck, Darmstadt) and the solvent system EtOAc-MeOH-H_2O (7:2:1). For a typical extraction procedure, fresh *Rauwolfia* cells (15 g) were extracted twice with MeOH (30 ml) under reflux for 20 min. After centrifugation (20 min, 4500×*g*), the supernatant was concentrated and chromatographed on tlc with the above-mentioned solvent system. Mass spectra were recorded under fab conditions with glycerol as matrix on a Finigan MAT 90 instrument. Nmr data were obtained on AM 400 and ARX 400 Bruker instruments; the solvent was DMSO-d_6–pyridine-d_5–D_2O (ca. 1:1:0.02). Ir spectra were recorded on a Perkin-Elmer 1310 spectrophotometer.

p-Hydroxyphenyl-O-β-D-*primeveroside* [**1**].—Amorphous solid which resisted crystallization: ir (KBr) 3440, 2974, 2862, 1635, 1573, 1459, 1385, 1267, 1223, 1171, 1100, 1078, 1049, 837, 738 cm^{-1}; fabms (glycerol) *m/z* (%) $[M-H]^-$ 403.5 (41), 349.5 (67), 293.4 (27), 265.4 (85), 255.4 (20), 183.2 (100), 151.2 (22); ^{13}C nmr δ (DMSO-d_6–pyridine-d_5–D_2O) 152.51 (C-4), 150.75 (C-1), 118.16 (C-3, C-5), 115.96 (C-2, C-6), 104.33 (C-1″), 102.24 (C-1′), 76.80 (C-3′), 76.70 (C-3″), 76.10 (C-5′), 73.73 (C-2″), 73.64 (C-2′), 70.10 (C-4′), 69.96 (C-4″), 68.76 (C-6′), 65.91 (C-5″); ^1H nmr δ (DMSO-d_6–pyridine-d_5–D_2O) 7.01 (d, 2H, J=8.9, H-3, H-5), 6.78 (d, 2H, J=8.9, H-2, H-6), 4.80 (d, 1H, J=7.5, H-1′), 4.36 (d, 1H, J=7.6, H-1″), 4.12 (dd, 1H, $J_{HA\text{-}6'/HB\text{-}6'}$=11.4, $J_{HA\text{-}6'/H\text{-}5'}$=1.2, HA-6′), 3.79 (dd, 1H, $J_{He\text{-}5''/Ha\text{-}5''}$=11.2, $J_{He\text{-}5''/H\text{-}4''}$=5.3, H_e-5″), 3.72 (dd, 1H, $J_{HB\text{-}6'/HA\text{-}6'}$=11.4, $J_{HB\text{-}6'/H\text{-}5'}$=5.2, HB-6′), 3.62 (ddd, 1H, $J_{H\text{-}5'/H\text{-}4'}$=9.5, $J_{H\text{-}5'/HB\text{-}6'}$=5.2, $J_{H\text{-}5'/HA\text{-}6'}$=1.2, H-5′), 3.50 (ddd, 1H, $J_{H\text{-}4''/Ha\text{-}5''}$=10.3, $J_{H\text{-}4''/H\text{-}3''}$=8.8, $J_{H\text{-}4''/He\text{-}5''}$=5.3, H-4″), 3.47 (dd, 1H, $J_{H\text{-}3'/H\text{-}2'}$=9.4, $J_{H\text{-}3'/H\text{-}4'}$=8.8, H-3′), 3.44 (dd, 1H, $J_{H\text{-}2'/H\text{-}3'}$=9.4, $J_{H\text{-}2'/H\text{-}1'}$=7.5, H-2′), 3.41 (dd, 1H, $J_{H\text{-}4'/H\text{-}5'}$=9.5, $J_{H\text{-}4'/H\text{-}3'}$=8.8, H-4′), 3.32 (dd, 1H, $J_{H\text{-}3''/H\text{-}4''}$=$J_{H\text{-}3''/H\text{-}2''}$=8.8, H-3″), 3.21 (dd, 1H, $J_{H\text{-}2''/H\text{-}3''}$=8.8, $J_{H\text{-}2''/H\text{-}1''}$=7.6, H-2″), 3.09 (dd, 1H, $J_{Ha\text{-}5''/He\text{-}5''}$=11.2, $J_{Ha\text{-}5''/H\text{-}4''}$=10.3, Ha-5″).

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