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STRUCTURE OF THE WATER-SOLUBLE FEEDING STIMULANT FOR *SCOLYTUS MULTISTRIATUS*: A REVISION

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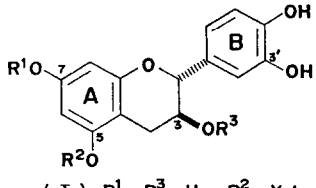
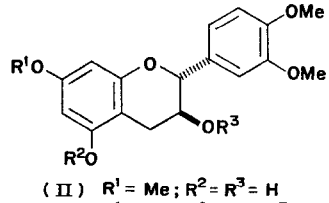
Key Word Index—*Ulmus americana*; Ulmaceae; elm; glycoside; catechinxyloside; feeding stimulant.

Abstract—The feeding stimulant for the smaller European elm bark beetle has been revised to (+)-catechin 7-β-D-xylopyranoside (III) from the structure (I) bearing the sugar at the 5-position.

TWO FEEDING stimulants, the wax lupeyl cerotate and the water-soluble glycoside catechin xyloside, which initiate feeding in the smaller European elm bark beetle, *Scolytus multistriatus* Marsham, were isolated from the host tree *Ulmus americana* L.[1] The glycoside was formulated as (+)-catechin 5-β-D-xylopyranoside (I) on the basis that the phenolic trimethyl ether of (+)-catechin derived from (I) gave a positive Gibbs' test[2] (positive for phenols with an unsubstituted *para* position), thus requiring that the phenolic group be at position 5 as in (II), since ring-B was fully methylated as shown by MS data. Evidence is presented herein requiring that the structure be revised to (+)-catechin 7-β-D-xylopyranoside (III). In addition, details are given for the preparation of related compounds, which were not possible in the first report.[1]



(I) $R^1 = R^3 = H$; $R^2 = Xyl$
(III) $R^1 = Xyl$; $R^2 = R^3 = H$
(IV) $R^1 = Xyl$; $R^2 = H$; $R^3 = Ac$
(VIII) $R^1 = Ara$; $R^2 = R^3 = H$



(II) $R^1 = Me$; $R^2 = R^3 = H$
(V) $R^1 = Xyl$; $R^2 = Me$; $R^3 = H$
(VI) $R^1 = R^3 = H$; $R^2 = Me$
(VII) $R^1 = Me$; $R^2 = R^3 = H$
(IX) $R^1 = Ara$; $R^2 = Me$; $R^3 = H$
(X) $R^1 = Xyl$; $R^2 = Me$; $R^3 = Ac$
(XII) $R^1 = triAc\text{-}Xyl$; $R^2 = Me$; $R^3 = Ac$

The isolation of catechin xyloside, an amorphous product, required the preparation of the crystalline heptaacetate in the purification scheme followed by saponification to regenerate the feeding stimulant. An improved method was devised for the saponification which gave a cleaner product with no contamination by the 3-acetate (IV). Separation of

[1] DOSKOTCH, R. W., CHATTERJI, S. K. and PEACOCK, J. W. (1970) *Science* **167**, 380.
[2] KING, F. E., KING, T. J. and MANNING, L. C. (1957) *J. Chem. Soc.* 563.

the two products (II) and (IV), however, can be performed on a polyamide column. The 3-acetate surprisingly lacks the feeding stimulant activity,[3] since simple phenolics in general have been reported to be active.[4]

Methylation of the homogeneous catechin xyloside by prolonged[5] diazomethane treatment afforded the trimethyl catechin xyloside (V), m.p. 191–193°, which was hydrolyzed and the product, 5,3′,4′-trimethyl-(+)-catechin (VI), rigorously purified. A negative Gibbs' test was now obtained with this material. However, both 5,3′,4′-trimethyl-(+)-catechin (VI) and 7,3′,4′-trimethyl-(+)-catechin (VII) have been reported,[6] but our material showed physical constants different from either. Similar lack of agreement was observed with the acetate derivative. The known compounds (VI) and (VII) were unavailable from the investigators for a direct comparison, so a direct approach was necessary.

The structure of polydine (VIII), the only other reported natural catechin glycoside (a catechin arabinoside), was established on the basis that its amorphous trimethyl ether (IX) on hydrolysis gave 5,3′,4′-trimethyl-(+)-catechin identical with an authentic sample.[6] On repeating this work, our crystalline trimethyl polydine (IX), m.p. 119–121°, gave the same trimethyl-(+)-catechin obtained from catechin xyloside. The acetates were also identical.

Previous supporting evidence[1] for assigning the anomeric carbon of catechin xyloside the β-configuration was the hydrolysis by the almond β-glucosidase, emulsin.[7] Additional support was obtained by application of Hudson's isorotation rule as used by Klyne,[8] for which the calculated $[M]_D$ for (+)-catechin α-D-xylopyranoside and β-D-xylopyranoside are +296° and −62°, respectively. The $[M]_D$ of the natural product is −142°, which is in agreement with the latter anomer.

EXPERIMENTAL

M.ps are uncorrected. IR spectra were determined in $CHCl_3$ or in KBr pellets, UV spectra in MeOH. NMR spectra were in $CDCl_3$ or as stated otherwise with TMS as internal standard and chemical shifts reported in δ (ppm) values. MS were obtained from a MS-9 high resolution mass spectrometer at 70 eV. TLC was performed on silica gel G with H_2SO_4–Et_2O (1:4) as spray reagent. Analyses were courtesy of A. H. Robins Co., Richmond, Virginia.

Hydrolysis of catechin xyloside heptaacetate. A solution of 9 g of the heptaacetate[1] in 450 ml MeOH was refluxed on a steam bath under N_2, and 1·0 l. of 0·1 N KOH in MeOH added over 30 min. Refluxing continued 1·5 hr more, and after cooling 210 ml AG 50W–X4 (H^+) resin in MeOH was added under N_2 while stirring. The filtrate was evaporated and azeotroped with C_6H_6 to give 5·1 g of amorphous (+)-catechin β-D-xylopyranoside (III), $[\alpha]_D^{22}$ −33·7° (*c* 0·126, H_2O). (Found: C, 52·0; H, 5·1. $C_{20}H_{22}O_2.2H_2O$ requires: C, 52·4; H, 5·7%.) TLC R_f 0,30 EtOAc–MeCOEt–HOAc–H_2O (12:4:1:1).

(+)-*Catechin-β-D-xylopyranoside 3-acetate* (IV). A solution of 2·5 g of catechin xyloside heptaacetate[1] in 1 l. of refluxing MeOH and under N_2 was treated over a 10-min period with 1·2 g KOH in 250 ml MeOH. After refluxing 15 min more, 70 ml AG 50W–4X (H^+) resin in MeOH was added under N_2; the mixture was cooled, filtered and the filtrate evaporated to give 1·4 g of residue containing catechin xyloside and the 3-acetate. The residue (1·2 g) was separated on a column of nylon 66 powder (80 g, 100 mesh) eluted first with MeOH–H_2O (1:9) and then with gradually increasing amounts of MeOH to MeOH–H_2O (1:1). Column fractions were followed by TLC; R_f 0·43 for the 3-acetate in EtOAc–MeCOEt–HOAc–H_2O (12:4:1:1). The yield of catechin xyloside (III) was 0·35 g and the 3-acetate (IV) 0·53 g $[\alpha]_D^{22}$ −69° (*c* 0·13, MeOH). (Found: C, 55·1; H, 5·3. $C_{22}H_{24}O_{11}$.MeOH requires: C, 55·6; H, 5·7%.) IR (KBr) 1712 cm^{-1} (AcO) and NMR (CD_3OD) δ 1·95 (Ac).

[3] In a test with 200 beetles, according to the procedure in Ref. 1, only 18 beetles showed a feeding response which was not different from the control as compared to catechin xyloside which elicites feeding in 80 insects.

[4] BAKER, J. E., RAINEY, D. P., NORRIS, D. M. and STRONG, F. M. (1968) *Forest Sci.* **14**, 91.

[5] This was absolutely necessary to completely methylate the 3′-phenolic group which is known to be the least reactive [SZABO, V., LITKEI, GY., FARKAS, E. and BOGNAR, R. (1967) *Acta Phys. Chim. Debrecina* 145].

[6] WEINGES, K. and WILD, R. (1970) *Ann. Chem.* **734**, 46.

[7] HELFERICH, B., GUNTHER, E. and PIGMAN, W. W. (1939) *Chem. Ber.* **72B**, 1953.

[8] KLYNE, W. (1950) *Biochem. J.* **47**, 41.

5,3′,4′-Trimethyl-(+)-catechin-7-β-D-xylopyranoside (V). A sample (5 g) of catechin xyloside (III) in 80 ml of MeOH at 0° was treated with excess CH_2N_2 for 113 hr. The residue after removal of solvent was purified on a column of silica gel G (300 g) with $CHCl_3$–MeOH (9:1) as eluent and the effluent residue crystallized from MeOH–Et_2O to give 2·7 g of the trimethyl ether (V), m.p. 193–194°, $[\alpha]_D^{22}$ −39° (*c* 0·51, MeOH). (Found: C, 59·3; H, 6·2; MW (MS) 464·1690 (2%). $C_{23}H_{28}O_{10}$ requires: C, 59·5; H, 6·1%; MW 464·1682) and NMR (CD_3OD) δ 3·79 (MeO), 3·82 (2 MeO).

5,3′,4′-Trimethylpolydine (IX). Polydine (300 mg) dissolved in 4 ml of MeOH was treated with ethereal CH_2N_2. The reaction residue was chromatographed on a column of silica gel G (30 g) with $CHCl_3$–MeOH (9:1) as solvent, and crystallization from MeOH–Et_2O to give 0·19 g of trimethyl ether (IX), m.p. 119–121°, $[\alpha]_D^{22}$ −73·5° (*c* 0·119, MeOH). (Found: C, 58·8; H, 6·2. $C_{23}H_{28}O_{10}.0{\cdot}5$ MeOH requires: C, 58·7; H, 6·3%), NMR (Pyr-d_f) δ 3·68, 3·73 and 3·76 (1 MeO each) and TLC R_f 0·24 in $CHCl_3$–MeOH (9:1).

5,3′,4′-Trimethyl-3-acetyl-(+)-catechin-7-β-D-xylopyranoside (X). Crystallization of the mother liquor residue from trimethylcatechin xyloside (V) (if any of the 3-acetate was a contaminant) gave from Et_2O–EtOAc the 3-acetate (X): m.p. 109°, $[\alpha]_D^{22}$ −24° (*c* 1·83, MeOH), [MW (MS) 506·1711 (0·7%). $C_{25}H_{30}O_{11}$ requires MW 506·1788], IR ($CHCl_3$) 1730 cm^{-1} (AcO), and NMR (CD_3OD) δ 4·89 (1H, *d*, 5·1, H_1 of xylose), 3·81, 3·78 and 3·76 (1 MeO each).

5,3′,4′-Trimethyl-(+)-catechin-β-D-xylopyranoside tetracetate (XI). The acetylation of either compound (V) or (X) gave from EtOH as needles (XI) m.p. 119–121°, $[\alpha]_D^{23}$ −14° (*c* 0·52, $CHCl_3$). (Found: C, 58·6; H, 5·7. $C_{31}H_{36}O_{14}$ requires: C, 58·9; H, 6·0%.) IR ($CHCl_3$) 1745 cm^{-1} (AcO), and NMR ($CDCl_3$) δ 3·88 (2 MeO), 3·78 (MeO), 2·08, 2·07, 1·95 (1 AcO each).

5,3′,4′-Trimethyl-(+)-catechin (VI). (a) *From trimethylcatechin xyloside* (V). A 500-mg sample of glycoside (V) was refluxed in 10 ml 0·2 N HCl in MeOH for 2 hr. After addition of 20 ml MeOH–H_2O (1:1) and cooling (0°) the ppt. was collected and crystallized from MeOH to give 202 mg of aglycone (VI), m.p. 258–260° (decomp.) $[\alpha]_D^{23}$ + 65° (*c* 0·40, C_5H_5N) [lit. value[6] m.p. 246°, $[\alpha]_{578}^{20}$ + 10·5° (*c* 2, Me_2SO)]. (Found: C, 64·7; H, 6·2. $C_{18}H_{20}O_6$ requires: C, 65·1; H, 6·1%) and NMR (CD_3SOCD_3) δ 3·80 (2 MeO) and 3·76 (MeO). The diacetate of (VI) showed m.p. 99–101°, $[\alpha]_D^{22}$ − 34° (*c* 0·08, Me_2CO) [lit. value[6] m.p. 124–125°, $[\alpha]_{578}^{20}$ + 2·5° (*c* 2, Me_2CO)] (C, 63·4; H, 5·9. $C_{22}H_{24}O_8$ requires: C, 63·5; H, 5·8%) and was identical with the sample prepared from trimethylpolydine (IX). From the filtrate of the reaction mixture was isolated the methyl α- and β-xylopyranosides and identified by direct comparison (m.p., IR, optical rotation) with authentic samples.

(b) *From trimethylpolydine* (IX). A 12-mg sample of trimethylpolydine was hydrolyzed as described for glycoside (V) to yield 5 mg of 5,3′,4′-trimethyl-(+)-catechin identical with (m.p., m.m.p., IR, UV, TLC, and NMR) a sample from trimethylcatechin xyloside (V).

Methylation of 5,3′,4′-trimethyl-(+)-catechin (VI). A sample of (VI) (40 mg) was suspended in 10 ml MeOH and treated with excess CH_2N_2 to give 5,7,3′,4′-tetramethyl-(+)-catechin (16 mg from EtOH). The product was identical (m.p., m.m.p., IR, TLC, and NMR) with a sample prepared from (+)-catechin.

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