Isolation and Structure of a New Phenolic Glycoside, Neocretanin, from Chestnut Galls[†]

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A tannic substance named neocretanin was isolated from chestnut galls by chromatographic separation. The chemical structure of neocretanin was established as 1-O-(2',6'-dihydroxy-4'-formyl)-phenyl-6-O-galloyl- β -D-glucopyranose (I) on the basis of spectral analyses and enzymatic hydrolyses.

In a series of biochemical studies on chestnut galls, we have isolated several kinds of phenolic constituents.^{1,2)} These are mainly phenolic glucosides constituted of 3,4,5-trihydroxybenzyl alcohol, gallic acid or dehydrodigallic acid. The present paper deals with the structure of a newly isolated related tannic substance which is named neocretanin. On this occasion, we propose the names "cretanin" and "crenatin" for the compounds MP-2 and MP-10, respectively, whose structures were elucidated in the previous paper.²⁾

In the isolation process of crenatin, a crystalline phenolic constituent was found in small quantity in the fraction E' of Sephadex LH-20 column chromatography of the gall extracts.¹⁾ This compound, neocretanin, was isolated from a relatively large amount of galls (112.7 kg) collected over two seasons by a procedure similar to that described pre-

viously.¹⁾ Sephadex LH-20 column chromatography using 50% methanol as solvent was effective for the separation of neocretanin, which was obtained as colorless needles, mp 195~197°C (decomp.), C₂₀H₂₀O₁₃·2H₂O. The IR spectrum is given in Fig. 1. Hydrolysis of

FIG. 1. IR Spectrum of Neocretanin in KBr Disk.

neocretanin with crude tannase preparation⁵⁾ yielded gallic acid and D-glucose, which were identified by paper chromatography (PC), and an unknown phenolic compound (IV). However, on hydrolysis with purified tannase neocretanin produced gallic acid and a new compound, named neocrenatin (V). By subsequent treatment of the digest with β -glucosidase, neocretanin was readily hydrolyzed to D-glucose and compound IV. Neocretanin, neocrenatin and compound IV showed an intense yellow color by spraying with aniline

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hydrogen phthalate. Similarly, *p*-hydroxybenzaldehyde, *o*-vanillin, vanillin, and 3,4-dihydroxybenzaldehyde gave positive color reaction with the same reagent. The results suggested that neocretanin, neocrenatin and compound IV possess an *ortho*- or *para*-substituted benzaldehyde structure in their molecule. Reduction of neocretanin with sodium borohydride gave compound III, *R*_f 0.35 (2% AcOH), 0.28 (BuOH-AcOH-H₂O, 4:1:5), which showed negative color reaction with the above reagent. Subsequent hydrolysis of the compound III with purified tannase yielded gallic acid and crenatin, which were identified by PC. The latter product was characterized by dark red color reaction with diazotized benzidine reagent. Consequently, neocrenatin (V) could be presumed to be 2,6-dihydroxy-4-

formylphenyl- β -D-glucoside.

The next problem to be solved in elucidating the complete structure of crenatin is the determination of the linking position of gallic acid to neocrenatin (V). Mass spectrometry of the peracetyl derivative II gave suggestive information. The major and important fragmentation pattern of II is illustrated in Scheme 1. The first prominent peak at *m/e* 567 and the peak at *m/e* 238 were attributed to triacetyl-(triacetyl-galloyl)-glucose oxonium and 3,5-diacetoxy-4-hydroxybenzaldehyde ion due to primary fragmentation at the C-1 bond of the glucose, respectively. Accordingly, the galloyl group should be linked to the glucose moiety. If the galloyl group were linked to hydroxybenzaldehyde moiety, peaks of *m/e* 474, corresponding to the aglycone

SCHEME 1.

and m/e 331, characteristic of peracetyl glucoside having a substituent only at C-1 of the glucose molecule, would be found instead of that of m/e 567. The peaks at m/e 405 and 279 are attributable to the loss of two molecules of acetic acid and one molecule of ketene from the peaks at m/e 567 and 441, respectively. These fragmentations take place much more readily when all three acetyl groups are continuously arranged in the glucose moiety.³⁾ Therefore, the galloyl group should be linked to C-6 of the glucose.

The PMR spectrum of II showed two singlets, at δ 2.03 (3H) and 2.06 (6H), due to three sugar acetyl groups. The singlet at δ 2.30 (15H) is assigned to five aromatic acetyl groups. The sugar protons are detected at δ 4.16 (1H, m), 4.49 (2H, m) and 5.0~5.5 (4H, m). The singlets at δ 7.43 (2H) and 7.82 (2H) are attributed to aromatic protons of hydroxybenzaldehyde and galloyl moiety, respectively. The formyl proton is observed at δ 9.80 (1H, s).

Thus, the structure of neocretanin was concluded to be 1-O-(2',6'-dihydroxy-4'-formyl)-phenyl-6-O-galloyl- β -D-glucopyranose (I). It is obscure whether the intact galls contain the compound in the benzaldehyde or benzyl alcohol form or both, but the isolation of the compound from a natural source has never been reported.

EXPERIMENTAL

All melting points were uncorrected. UV spectra were measured on a Shimadzu MPS-50 spectrophotometer and IR spectra with a JASCO IRA-1 spectrophotometer. Optical rotations were determined on a JASCO DIP-SL polarimeter. PMR spectra were measured on a JEOL JNM-MH-100 spectrometer with TMS as internal standard. Mass spectra were obtained with a Hitachi RMS-4 spectrometer equipped with a direct inlet system. The electron accelerating energy was 70 eV and chamber temperature 200°C. Paper chromatography was carried out on Toyo Roshi No. 5B papers with the following solvents: (A) 2%

AcOH, (B) BuOH-AcOH-H₂O (4:1:5, upper layer, BAW). Phenolic compounds on the chromatograms were detected under UV light and by spraying (1) 0.5% iron alum, (2) 0.5% potassium ferricyanide, and (3) diazotized benzidine. TLC was accomplished on silica gel plates (Wakogel B-5F).

Isolation and some properties of neocretanin

Fractions E and F of silica gel column chromatography were prepared as described in the previous paper¹⁾ from the galls (112.7 kg) collected over two seasons and further applied on a column of Sephadex LH-20 (7×32 cm) equilibrated with 50% methanol. The column was washed with the same solvent at a flow rate of 150 ml per hour and the effluent was collected in 18-ml fractions. The fractions were monitored by UV and PC. Neocretanin was eluted in the fraction number 230~280 with a few other phenolics. The fractions were combined and concentrated to a small volume of dark brown aqueous solution and then left in a refrigerator for several days. Resultant crude crystals (44 mg) were collected by filtration and recrystallized from water after treatment with decolorizing carbon to give colorless needles (40 mg). The compound was insoluble in hexane, benzene and chloroform, slightly soluble in acetone, ethyl acetate and ether, soluble in water, and readily soluble in methanol. Reaction with 0.5% gelatin as well as with neutral lead acetate gave a white precipitate, and all other tests for tannic substances were also positive. Mp 195~197°C (decomp.), $[\alpha]_D^{25} - 63.8^\circ$ ($c=0.99$, MeOH). Anal. Found: C, 47.84; H, 4.60. Calcd. for C₂₀H₂₀O₁₃·2H₂O: C, 47.61; H, 4.76%. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 284 (15900), UV λ_{max}^{alkali} nm: 250, 290, 360, 390(sh). IR ν_{max}^{KBr} cm⁻¹: 3500~3200, 1675, 1610, 1590, 1530, 1450, 1410, 1335, 1280, 1225, 1160, 1120, 1075, 940, 900, 880, 850, 835, 760, 730 (Fig. 1). Rf values on PC: 0.30 (2% AcOH), 0.36 (BAW). Color reaction with aniline hydrogen phthalate showed yellow color without heating.

Enzymatic hydrolysis

Neocretanin (1 mg) was dissolved in 0.25 ml of M/30 phosphate buffer (pH 5.5). A very small volume of purified tannase concentrate⁴⁾ was added to the solution and the mixture was kept at 30°C for one hour, then a portion of the reaction mixture was subjected to two-dimensional PC. PC analyses indicated that neocretanin was hydrolyzed to gallic acid, Rf 0.40 (2% AcOH), 0.62 (BAW) and a new compound, named neocretanin (V), showing Rf 0.74 (2% AcOH), 0.33 (BAW). By subsequent treatment of the digest with β -glucosidase (emulsin), V was hydrolyzed to D-glucose, Rf 0.18 (BAW), 0.39 (phenol saturated with water) and phenolic compound IV, 3,4,5-trihydroxybenzaldehyde, Rf 0.50 (2% AcOH), 0.70 (BAW).

Hydrolysis of neocretanin with crude tannase preparation gave gallic acid, D-glucose and compound IV. Neocretanin and compound IV showed yellow color reaction with aniline hydrogen phthalate.

Acetylation

Neocretanin (19 mg) was dissolved in a mixture of acetic anhydride (0.5 ml) and pyridine (0.2 ml) by heating for 10 min at 80°C. The reaction mixture was allowed to stand overnight at room temperature, then poured into 10 ml of ice-water. The white precipitate (29 mg) formed was collected and crystallized from ethanol to give colorless wooly needles (II). Yield 21 mg, mp 139~140°C, $[\alpha]_D^{25}$ 4.63° ($c=1.30$, MeOH). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1765, 1740, 1695, 1590, 1480, 1420, 1360, 1315, 1200, 1050, 890, 850, 805, 750. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 2.03, 2.06 (9H, s, 3 × OCOCH₃), 2.30 (15H, s, 5 × OCOCH₃), 4.16 (1H, m, sugar proton), 4.49 (2H, m, sugar protons), 5.0~5.5 (4H, m, sugar protons), 7.43 (2H, s, Ar-H), 7.82 (2H, s, Ar-H), 9.80 (1H, s, CHO). MS m/e : 567(8), 525(7), 507(0.3), 483(6), 465(0.6), 441(2), 423(1), 405(12), 381(1), 363(7), 339(0.9), 321(7), 279(75), 238(25), 237(100), 196(30), 195(73), 169(36), 154(38), 153(65), 152(52), 127(21), 125(9), 109(51). Anal. Found: C, 53.66; H, 4.40. Calcd. for C₃₆H₃₀O₂₁: C, 53.73; H, 4.48%. Rf value on TLC: 0.33 (Bz-acetone, 5:1).

Reduction of neocretanin

Neocretanin (2 mg) was dissolved in dioxane (0.2 ml) and sodium borohydride (10 mg) suspended in dioxane

(0.5 ml) was added to the solution. The solution was heated for 7 hr at 80°C, then the solvent was distilled off under reduced pressure. The concentrate was dissolved in 2 N hydrochloric acid (0.2 ml) and extracted sufficiently with ethyl acetate. Concentration of the extract gave brown powdery reduction product (III) (1.7 mg) showing Rf 0.35 (2% AcOH), 0.28 (BAW). The color reaction of III with aniline hydrogen phthalate was negative.

Hydrolysis of III with tannase

Reduction product III (1.7 mg) was dissolved in phosphate buffer (0.5 ml) and hydrolyzed with purified tannase as described above. PC analyses of the hydrolysate indicated the presence of gallic acid, Rf 0.40 (2% AcOH), 0.62 (BAW) and crenatin, Rf 0.74 (2% AcOH), 0.37 (BAW). The latter substance gave dark red color, characteristic of the authentic crenatin,¹⁾ with diazotized benzidine.

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