Journal of Natural Products
Vol. 53, No. 5, pp. 1297-1301, Sep-Oct 1990

TANNINS AND RELATED COMPOUNDS, 101.[1] ISOLATION AND STRUCTURES OF *C*-GLYCOSYL HYDROLYZABLE TANNINS FROM TURKISH GALLS

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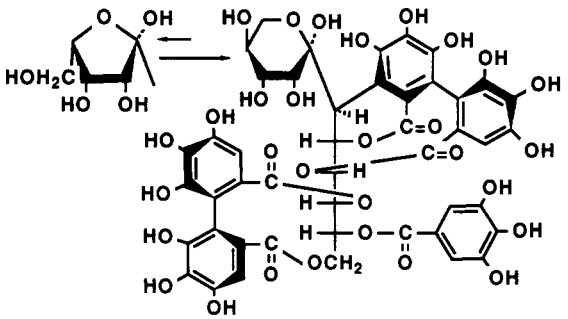
ABSTRACT.—A chemical examination of Turkish galls (*Quercus lustitanica*) has led to the isolation of four *C*-glycosidic hydrolyzable tannins, among which three were found to be identical with the known compounds pterocarinin A [**1**], castalagin [**2**], and castavaloninic acid [**4**]. The structure of the new tannin, isocastavaloninic acid [**5**], was established on the basis of spectroscopic and chemical evidence. The complete assignment of the structure of castavaloninic acid [**4**], including the absolute stereostructure and the orientation of the valoneayl group, is also described.

Turkish galls (formed on *Quercus lusitanica* Lamarck) are one of the important crude drugs used for the preparation of commercial tannic acids. The presence of large quantities of gallotannins in the extract has long been known, and our previous work demonstrated that they consist of a mixture 1,2,3,6-tetra-*O*- and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose cores with one to four depside galloyl groups (1). The co-existence of ellagitannins in the galls was suggested much earlier by the isolation of ellagic acid (2). There is, however, no chemical work on the hydrolyzable tannins other than gallotannins. We now report the isolation and characterization of two ellagitannins **1** and **2** and two related compounds **4** and **5**.

RESULTS AND DISCUSSION

A large amount of the gallotannins present in the aqueous Me_2CO extract was efficiently removed by partition with H_2O and EtOAc (3). The H_2O layer, which exhibited a positive $NaNO_2$-HOAc test (4), was repeatedly chromatographed over Sephadex LH-20 and various reversed-phase gels (5,6) to afford four tannins **1**, **2**, **4**, and **5**. Compounds **1**, **2**, and **4** were found to be identical with pterocarinin A (7), castalagin (8–10), and castavaloninic acid (11), respectively, by comparisons of their physical and spectral data with those of authentic samples.

Because the absolute stereostructure of castavaloninic acid [**4**], as well as the orientation of the valoneaic acid ester group located at the *C*-glycosyl 4,6 positions, was still unsolved (11), we first attempted to elucidate these. Ordinary phenol methylation of **4**



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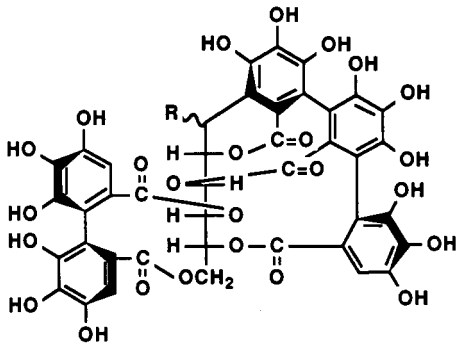
[1]For Part 100 in this series, see T. Tanaka, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, in press.



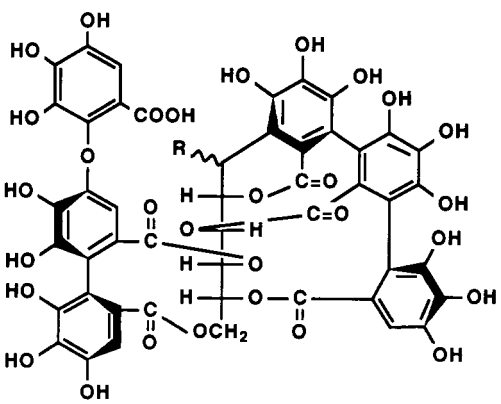
2 R= ⋯OH
3 R= —OH

yielded the octadecamethyl ether, which was subsequently subjected to mild alkaline methanolysis to give products **6** and **8**. The specific optical rotation and the ^{1}H-nmr spectrum of **6** were identical with those of the sample prepared similarly from castalagin [**2**], thus establishing the chirality of the triphenoyl group to be in the *S,S* series (8–10). The product **8** was found to be trimethyl octa-*O*-methylvaloneate, and the negative sign of the specific optical rotation indicated that the atropisomerism of the biphenyl bond is in the *S* series (12). Comparison of the ^{1}H-nmr spectra (Table 1) of **2**

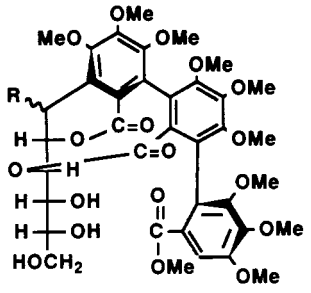
TABLE 1. ^{1}H-nmr Data for *C*-Glycosyl Protons in Compounds **2**–**5** (Me_2CO-d_6 + D_2O, ppm, *J* in Hz).

Proton	Compound			
	2[a]	**3**[a]	**4**[b]	**5**[b]
H-1	5.72 d (5)	4.92 d (2)	5.45 d (5)	4.87 d (2)
H-2	5.05	5.30 dd (2,2)	4.75 dd (5,2)	5.13 dd (2,2)
H-3	5.05	4.59 dd (2,7)	4.93 dd (2,7)	4.57 dd (2,7)
H-4	5.27 t (7)	5.23 t (7)	5.25 t (7)	5.20 t (7)
H-5	5.61 dd (7,3)	5.66 dd (8,3)	5.56 dd (7,3)	5.62 br d (7)
H-6	4.05 d (13)	4.08 d (13)	4.00 d (13)	4.05 d (13)
	5.09 dd (13,3)	5.08 dd (13,3)	5.10 dd (13,3)	5.01 dd (13,3)

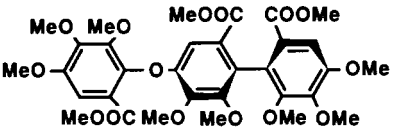
[a]400 MHz.
[b]270 MHz.



4 R= ⋯OH
5 R= —OH



6 R= ⋯⋯OH
7 R= ——OH



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and **4** showed that the glucosyl H-1 and H-2 signals in **4** were shifted upfield by ca. 0.3 ppm. This could be interpreted in terms of the anisotropic effect of the "branched" gallic acid aromatic ring; therefore, coupled with the Dreiding model examination, the orientation of the valoneayl group was concluded to be as represented by the formula **4**.

The negative fabms (13) of compound **5** (named isocastavaloninic acid) showed the same prominent $[M-H]^-$ peak at *m/z* 1101 as that of castavaloninic acid [**4**]. The absence of a hemiacetal carbon signal in the ^{13}C-nmr spectrum (Table 2) indicated compound **5** to be a *C*-glycosidic hydrolyzable tannin. A small coupling constant of the H-1 signal (Table 1) suggested that **5** possesses the α-hydroxyl group at C-1 (8–10), differing from those of castalagin [**2**] and castavaloninic acid [**4**]. Furthermore, the coupling patterns of the polyalcohol moiety were in good agreement with those of vescalagin [**3**], thus indicating that **5** is a C-1 epimer of castavaloninic acid [**4**].

Further structural confirmation was obtained as follows. Mild alkaline methanolysis of the octadecamethyl ether of **5** yielded, together with trimethyl octa-*O*-methyl-(*S*)-valoneate [**8**], product **7**, which was found to be identical with a sample obtained by similar treatment of vescalagin [**3**]. As for the orientation of the valoneayl group in **5**, the upfield shift (ca. 0.2 ppm) of the H-2 signal in **5**, as compared with that of **3**, indicated it to be the same as in **4**.

According to pharmacopoeias, tannic acids are regarded in North America and Europe to be produced from Turkish galls, whereas in Asian countries, they are prepared from the twig galls of several *Rhus* species (Chinese galls). Although they are from quite different origin, the nature of the tannic acids has generally been accepted to be al-

TABLE 2. ^{13}C-nmr Data for *C*-Glycosyl Carbons in Compounds **2–5** (Me_2CO-d_6 + D_2O, ppm).

Carbon	Compound			
	2[a]	**3**[a]	**4**[c]	**5**[b]
C-1	67.4	66.1	66.9	65.7
C-2	74.5	78.3	73.6	77.9
C-3	66.7	69.1	66.2	68.7
C-4	69.7	70.0	69.0	69.8
C-5	71.7	71.7	71.3	71.5
C-6	65.8	66.0	65.5	65.4

[a]100 MHz.
[b]67.5 MHz.
[c]25.05 MHz.

most the same in the fields of pharmacology and biochemistry, because they all consist of polygalloyl glucoses (1,3). We have recently found that there are remarkable differences in the biological activities between gallo- and other hydrolyzable tannins (14–16); therefore, the occurrence of the *C*-glycosyl hydrolyzable tannins in Turkish galls suggests that different biological activities would be expected in these two tannic acids, although the gallotannins (total yield 64%) predominate in the Turkish gall extract. Finally, it should be noted that of the four tannins isolated here, three (**1**, **2**, and **4**) are occasionally isolable as major metabolites from *Quercus* species (17–20).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a JASCO DIP-4 digital polarimeter (cell length 0.5 dm). ^{1}H- and ^{13}C-nmr spectra were taken with JEOL FX-100, GX-270, and GX-400 spectrometers in Me_2CO-d_6/D_2O (for phenolics) and $CDCl_3$ (for methyl ethers) with TMS as an internal reference. Fabms, eims, and fdms were recorded on JEOL D-300 and DX-300 spectrometers. Cc was carried out with Sephadex LH-20 (25–100μ, Pharmacia Fine Chemical), MCI-gel CHP 20P (75–150μ, Mitsubishi Chemical Industries), Fuji gel ODS G3 (43–65μ, Fuji gel Hanbai), and Kieselgel 60 (70–230 mesh, Merck). Tlc was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm thick, Merck) in the solvent systems C_6H_6-HCOOEt-HCOOH (1:7:1), C_6H_6-Me_2CO (3:1 and 4:1), and C_6H_6-EtOH (9:1) or precoated cellulose F_{254} plates (0.10 mm thick, Merck) using the solvent system 2% HOAc; spots were detected by their blue fluorescence under uv light and the 2% $FeCl_3$/EtOH or $NaNO_2$/HOAc spray.

MATERIAL.—Turkish galls were purchased from the market in Jakarta, Indonesia. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Kyushu University.

EXTRACTION AND ISOLATION.—The pulverized galls (2.7 kg) were extracted five times with 80% aqueous Me_2CO at room temperature. After concentration of the combined extracts under reduced pressure, the resulting precipitates, consisting mainly of waxes and resins, were removed by filtration. The filtrate was extracted with EtOAc, and the EtOAc layer was found to contain large amounts of gallotannins. The H_2O layer was chromatographed on MCI-gel CHP 20P with H_2O containing increasing proportions of MeOH to give two fractions. Rechromatography of the first fraction over Sephadex LH-20 with 60% aqueous MeOH gave pterocarinin A [**1**] (130 mg). The second fraction was subjected to a combination of chromatography over Fuji gel ODS G3 (30% aqueous MeOH) and Sephadex LH-20 (60% aqueous MeOH) to afford castalagin [**2**] (810 mg), castavaloninic acid [**4**] (130 mg), and isocastavaloninic acid [**5**] (270 mg). ^{1}H-nmr and ^{13}C-nmr results are in Tables 1 and 2, respectively.

METHYLATION OF **4**.—A mixture of **4** (200 mg), Me_2SO_4 (1.5 ml), and K_2CO_3 (1.5 g) in dry Me_2CO (20 ml) was heated under reflux for 2 h. After removal of the inorganic salts by filtration, the filtrate was concentrated and applied to a Si gel column. Elution with C_6H_6-Me_2CO (9:1) yielded the octadecamethylate as a white amorphous powder (156 mg): $[\alpha]^{24}D$ −178.2° (c = 1.0, Me_2CO), ^{1}H nmr (100 MHz) δ 3.42–4.13 (OMe), 4.64 (1H, d, J = 5 Hz, H-2), 4.87 (1H, d, J = 7 Hz, H-3), 5.13 (1H, d, J = 13 Hz, H-6), 5.14 (1H, d, J = 5 Hz, H-1), 5.19 (1H, t, J = 7 Hz, H-4), 5.65 (1H, dd, J = 7,2 Hz, H-5), 6.47, 6.80, 6.94, 7.33 (each 1H, s, aromatic H); fdms m/z $[M]^+$ 1354. Calcd for $C_{66}H_{66}O_{31}\cdot \frac{1}{2}H_2O$, C 58.10, H 4.92; found C 58.27, H 5.21%.

METHANOLYSIS OF THE METHYLATE.—A solution of the octadecamethyl ether (90 mg) in 2% NaOH/MeOH (9 ml) was kept at room temperature for 3.5 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) and subjected to Si gel chromatography. Elution with C_6H_6-Me_2CO (9:1) afforded trimethyl octa-*O*-methyl-(*S*)-valoneate [**8**] as a pale yellow amorphous powder (17 mg): $[\alpha]^{24}D$ −14.9° (c = 1.7, $CHCl_3$); ^{1}H nmr (100 MHz) δ 3.48, 3.57, 3.60, 3.67, 4.07 (each 3H, s, OMe), 3.78 (6H, s, OMe), 3.94, 3.98 (12H in total, each s, OMe), 6.92, 7.30, 7.35 (each 1H, s, aromatic H). Further elution with C_6H_6-Me_2CO (4:1) gave the methanolysate **6** as a white amorphous powder (36 mg): $[\alpha]^{24}D$ −66.3° (c = 0.3, $CHCl_3$); ^{1}H nmr (100 MHz) δ 3.51, 3.57, 3.70, 3.84, 3.93, 4.02, 4.05, 4.09 (OMe), 4.80–5.04 (2H, m, H-2, -3), 5.50 (1H, d, J = 4 Hz, H-1), 7.19 (1H, s, aromatic H). The $[\alpha]D$ and ^{1}H-nmr spectrum of this compound coincided with those of a sample prepared similarly from castalagin [**2**].

ISOCASTAVALONINIC ACID [**5**].—An off-white amorphous powder: $[\alpha]^{22}D$ −120.3° (c = 1.0, H_2O); ^{1}H nmr (270 MHz) δ 6.63, 6.78, 6.90 , 7.16 (each 1H, s, aromatic H); ^{13}C nmr (67.5 MHz) δ 107.5, 109.0, 109.9 (valoneayl C-3, -3′, -3″), 110.1 (triphenoyl C-6″), 114.7 (valoneayl C-2″), 114.9 (× 2), 115.3 (× 2), 115.5 (triphenoyl C-2, -2′, -2″, -6, -6″), 118.8 (valoneayl C-1′), 125.3 (valoneayl C-

2′), 125.6 (triphenoyl C-1), 125.9 (valoneayl C-2), 136.2, 136.6, 137.2, 137.9 (valoneayl C-5 and triphenoyl C-4, -4′, -4″), 138.5 (×2), 139.5, 139.8 (valoneayl C-4″, -5′, -5″, -6″), 143.6 (valoneayl C-1″), 144.5, 144.8, 144.9, 145.2, 145.5 (×3), 146.9, 147.5, (valoneayl C-4, -4′, -6, -6′ and triphenoyl C-3, -3′, -3″, -5, -5′, -5″), 164.1 165.3, 166.0, 166.8, 168.2, 169.0 (-COO-); ^{1}H nmr and ^{13}C nmr of sugar resonances see Tables 1 and 2; negative fabms *m/z* $[M-H]^-$ 1101. Calcd for $C_{48}H_{30}O_{31}\cdot H_2O$, C 51.47, H 2.80; found C 51.39, H 2.91%.

METHYLATION OF **5**.—A mixture of **5** (200 mg), Me_2SO_4 (1.5 ml), and K_2CO_3 (1.5 g) in dry Me_2CO (20 ml) was refluxed for 3 h. Workup as described above yielded the octadecamethyl derivative as a white amorphous powder (132 mg): $[\alpha]^{12}D$ −93.2° (*c* = 1.0, $CHCl_3$); ^{1}H nmr (100 MHz) δ 3.22–4.12 (OMe), 4.43 (1H, br d, *J* = 7 Hz, H-3), 4.75 (1H, br d, *J* = 11 Hz, H-6), 5.01 (2H, m, H-1, 2), 5.21 (1H, t, *J* = 8 Hz, H-4), 5.87 (1H, br d, *J* = 7 Hz, H-5), 6.47, 6.76, 6.85, 7.36, (each 1H, s, aromatic H). Calcd for $C_{66}H_{66}O_{31}$, C 58.54, H 4.91; found C, 58.52, H 4.66%.

METHANOLYSIS OF THE METHYLATE.—A solution of the octadecamethyl ether (70 mg) in 2% NaOH-MeOH (2 ml) was left standing at room temperature for 3 h. Workup as described above gave trimethyl octa-*O*-methyl-(*S*)-valoneate [**8**] (23 mg) and the methanolysate **7** as a white amorphous powder (37 mg): $[\alpha]^{22}D$ − 12.8° (*c* = 1.8, $CHCl_3$); ^{1}H nmr (100 MHz) δ 3.53–4.12 (OMe), 4.31 (1H, d, *J* = 2 Hz, H-1), 5.13 (1H, t, *J* = 2 Hz, H-2), 7.36 (1H, s, aromatic H). Compound **7** was identified as the product obtained from vescalagin [**3**] in a similar manner.

ACKNOWLEDGMENTS

We thank Mr. Y. Tanaka, Miss K. Soeda, and Mr. R. Isobe of the Faculty of Pharmaceutical Sciences, Kyushu University, for spectral measurements. We also thank Mr. S. Kasahara of P.T. Eisai, Indonesia, for the identification of the plant material.

LITERATURE CITED

1. M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 961 (1983).
2. E. Fischer and K. Freudenberg, *Chem. Ber.*, **47**, 2485 (1914).
3. M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 2963 (1982).
4. E.C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).
5. G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981).
6. F. Hashimoto, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **37**, 3255 (1989).
7. G. Nonaka, K. Ishimaru, R. Azuma, M. Ishimatsu, and I. Nishioka, *Chem. Pharm. Bull.*, **37**, 2071 (1989).
8. G. Nonaka, T. Sakai, T. Tanaka, K. Mihashi, and I. Nishioka, *Chem. Pharm. Bull.*, **38**, 2151 (1990).
9. W. Mayer, W. Gabler, A. Riester, and H. Korger, *Justus Liebigs Ann. Chem.*, **707**, 177 (1967).
10. G. Nonaka, K. Ishimaru, M. Watanabe, I. Nishioka, T. Yamauchi, and A.S.C. Wan, *Chem. Pharm. Bull.*, **35**, 217 (1987).
11. W. Mayer, W. Bilzer, and G. Schilling, *Justus Liebigs Ann. Chem.*, 876 (1976).
12. R. Saijo, G. Nonaka, I. Nishioka, I.-S. Chen, and T.-H. Hwang, *Chem. Pharm. Bull.*, **37**, 2940 (1989).
13. R. Isobe, T. Tanaka, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **37**, 1748 (1989).
14. S. Shirahata, H. Murakami, K. Nishiyama, I. Sugata, K. Shinohara, G. Nonaka, I. Nishioka, and H. Omura, *Agric. Biol. Chem.*, **49**, 1033 (1985).
15. F. Kiuchi, Y. Tsuda, K. Konda, H. Yoshimura, I. Nishioka, and G. Nonaka, *Chem. Pharm. Bull.*, **36**, 1796 (1988).
16. G. Nonaka, I. Nishioka, M. Nishizawa, T. Yamagishi, G.E. Dutschman, A.J. Bodner, R.E. Kilkuskie, Y.-C. Cheng, and K.-H. Lee, *J. Nat. Prod.*, in press.
17. G. Nonaka, H. Nishimura, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 163 (1985).
18. K. Ishimatsu, M. Ishimaru, G. Nonaka, K. Mihashi, Y. Iwase, and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 3312 (1988).
19. K. Ishimaru, M. Ishimatsu, G. Nonaka, K. Mihashi, Y. Iwase, and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 3319 (1988).
20. G. Nonaka, S. Nakayama, and I. Nishioka, *Chem. Pharm. Bull.*, **37**, 2030 (1989).

Received 16 March 1990