MALLOTUSINIC ACID AND MALLOTINIC ACID, NEW HYDROLYZABLE TANNINS FROM MALLOTUS JAPONICUS

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The bark and the leaf of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae), which are rich in tannin, have been used as remedies of gastric ulcer in Japan. We have isolated a new hydrolyzable tannin named mallotusinic acid $(\underline{1})$, along with geraniin $(\underline{2})^{1,2}$, as the main constituents in the leaf. Another new hydrolyzable tannin named mallotinic acid $(\underline{3})$ has also been detected.

The leaf was homogenized in a mixture of acetone and water, and concentrated solution was extracted with EtOAc. The EtOAc extract was fractionated by droplet countercurrent chromatography developing with n-BuOH-AcOH-H₂O (4:1:5) by ascending method. Mallotusinic acid contaminated by geraniin was obtained from the fractions moving after rutin and geraniin. Better separation of mallotusinic acid from geraniin was effected by regulating pH of the aqueous layer (pH 4.5, and then pH 2.0) upon the extraction with EtOAc. After repeated reprecipitation from a mixture of EtOAc and petroleum ether, mallotusinic acid was obtained as brownish yellow amorphous powder. This tannin, C48H30031.11H20, $[\alpha]_{D}^{15}$ -65.4° (c 0.5, MeOH), gave the characteristic color reaction of ellagitannin with NaNO₂-AcOH, like geraniin³, on the paper chromatogram which showed a single spot [R_f 0.30(7% AcOH); 0.46(n-BuOH-AcOH-H₂O, 4:1:5, upper)]. The PMR spectrum of 1 (90 MHz, CD₃COCD₃) shows signals corresponding to seven protons in the region of aromatic and vinyl protons [δ 7.27(2H,s), 7.19(s), 7.15(s), 7.03 (5/8H,s), 6.92(3/8H,s), 6.67(s), 6.50(5/8H,s) and 6.20(3/8H,d,J=1.5Hz)], besides the sugar protons and two peaks corresponding to a methine proton [§ 5.10(5/8H, s) and 4.89(3/8H,d,J=1.5Hz)]. When measured in the presence of D₂O, each one

of the protons in the region of aromatic and vinyl protons, and the methine proton were exhibited by two peaks of approximately equal area in analogous way to equilibrated geraniin².

Reaction of mallotusinic acid with o-phenylenediamine yielded yellow precipitate which was purified by reprecipitation from MeOH-CHCl₃ to afford amorphous powder, $C_{54}H_{34}O_{29}N_2 \cdot 7H_2O$, $[\alpha]_D^{15}$ -21° (c 0.5, MeOH), which was named phenazine 1 $(\underline{4})$. The PMR spectrum of phenazine 1 showed analogy to that of phenazine B obtained from geraniin^{1,2}, except the presence of an additional aromatic proton peak in the former. Phenazine 1 was hydrolyzed in boiling water to give a dark red precipitate which was identified with phenazine C (5) produced by hydrolysis of phenazine B¹. Concentration of the mother liquor followed by repeated reprecipitation from MeOH-CHCl₃ yielded an amorphous tannin, C34H26023, which showed positive color reaction of ellagitannin, and was named mallotinic acid (3). Mallotinic acid was methylated with diazomethane to give dodecamethyl derivative 6, C46H50023.3H20, m.p. 110.5-113°. Methanolysis of 6 in NaOMe-MeOH followed by fractionation by preparative TLC yielded methyl tri-0-methylgallate, and another product, $[\alpha]_{D}^{20}$ +17° (c 1.0, acetone), (m/e 660, M^+), whose PMR peaks were identical with those of trimethyl octa-0-methylvaloneaate (7), $[\alpha]_{578}^{20}$ -18.4°⁴, obtained from valonia tannin, and is regarded as the atropisomer of the latter compound. Glucose was detected among the products of hydrolysis of 4, by gas chromatography of trimethylsilyl derivative, and paper chromatography. Mallotinic acid showed analogy to corilagin¹ in the overall PMR spectral pattern except the presence of an additional aromatic proton in the former, and also in the upfield shift of H-2 (δ 3.94) and H-4 (δ 4.36) upon its production by hydrolysis of 4. Methyl undeca-0-methylmallotinate (6) showed analogy of fragmentation in the mass spectrum to that of nona-0-methylcorilagin, exhibiting m/e 758 [M - tri-0methylgallic acid]⁺, m/e 632 [octa-0-methylvaloneaic acid monomethyl ester]⁺ and m/e 212 [tri-0-methylgallic acid]⁺ ions. The location of galloyl group at C-1 of glucose in 3 was proved by hydrolysis of 3 in water at 80°, which gave a mixture composed of two main products, between which one was gallic acid, and another one gave positive color reactions of ellagitannin and reducing sugar



(aniline phthalate) on the paper chromatogram.

The presence of free hydroxyl groups at C-2 and C-4 in <u>3</u> was shown by methanolysis (NaOMe-MeOH) of methyl trideca-0-methylmallotinate (<u>8</u>) produced by the reaction of <u>6</u> with MeI-Ag₂O. The sugar fraction of the methanolyzate yielded by preparative PPC, 2,4-di-0-methylglucose which was identified by GC-MS of trimethylsilyl derivative, with the product of analogous treatment of nona-0methylcorilagin. The structure of mallotinic acid is therefore represented by 3. Mallotinic acid was detected by high pressure liquid chromatography of the leaf extract.

The orientation of 2',3',4'-trihydroxy-2,3,4-trioxo-1,2,3,4-tetrahydrodiphenoyl group in the molecule of $\underline{1}$ is regarded as identical with that in geraniin, i.e., the carboxycyclohexenetrione molety is bonded to 0-4, and the galloyl molety is bonded to 0-2 of glucose, based on the analogy of upfield shift of H-1 of glucose upon the formation of phenazine B (δ 6.56 in $\underline{2} + \delta$ 6.03)¹ and phenazine 1 (δ 6.50 in $\underline{1} + \delta$ 6.13). This analogy is also in accord with β -configuration of the galloyl group at C-1 of glucose in $\underline{1}$ and $\underline{3}$. The structure of mallotusinic acid is thus represented by $\underline{1}$.

The equilibration of mallotusinic acid exhibited by the PMR spectra is regarded as due to the epimerization at the methine carbon as shown by $\underline{la} \neq \underline{lb}$. The ¹³C-NMR spectrum of the equilibrated mallotusinic acid (25 MHz, CD_3COCD_3 -H₂O) exhibits, in analogous way to that of equilibrated geraniin, the epimers by the peaks at δ 45.9, 51.9 (methine); δ 63.5, 64.4; 63.7, 64.1; 66.2, 67.3; 70.5, 71.5; 72.9, 73.9; 91.1, 91.7 (glucose); δ 92.3, 92.5, 96.2, 108.9 (carbon having geminal diol); and δ 192.1, 194.9 (ketone). These CMR spectral data also indicate that two of the three ketones in mallotusinic acid are hydrated to form geminal diols in the same way as equilibrated geraniin.

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