Table II. Effects of Added Compounds on the Catalase-catalyzed Decomposition of H_2O_2

Additions to the catalase solution (1.4 μM)[a]	Activity[b]	% of control
Control	11100	100
+NaN_3 (0.4 mM)[a]	5200	47
+NaN_3 (2.0 mM)[a]	800	7.2
+Cumene hydroperoxide (1.5 mM)[a]	11100	100

a) The concentration in the mixture of catalase and additives. The mixture was allowed to stand for 5 min and then diluted with 0.05 M phosphate buffer (pH 7.0) for the assay.
b) Sigma units per mg protein. One Sigma unit will decompose one μmol of H_2O_2 per minute at pH 7.0 at 25°, while the H_2O_2 concentration falls from 10.3 to 9.2 μmol per ml of reaction mixture.

detected by spin trapping technique[6] and gas chromatography in our system. Therefore, the active site of catalase for the cumene hydroperoxide-supported N-demethylation is considered to be different from that for the decomposition of hydrogen peroxide. Detailed studies are now in progress.

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Received November 24, 1979

6) The spin traps used were 0.1 M phenyl-*tert*-butylnitrone and 0.1 M 5,5-dimethyl-1-pyrroline-1-oxide. E.G. Janzen and B.J. Blackburn, *J. Am. Chem. Soc.*, **91**, 4481 (1969); E.G. Janzen and J. I-Ping Liu, *J. Magn. Resonance*, **9**, 510 (1973); A.N. Saprin and L.H. Piette, *Arch. Biochem. Biophys.*, **180**, 480 (1977).

[Chem. Pharm. Bull. 28(2) 685—687 (1980)]

Eugeniin, a New Ellagitannin from Cloves

A new ellagitannin, eugeniin was isolated from cloves, the dried flower buds of *Eugenia caryophyllata* Thunb., and the structure was determined to be 1,2,3-trigalloyl 4,6-hexahydroxydiphenoyl β-D-glucopyranose.

Keywords——ellagitannin; eugeniin; clove; *Eugenia caryophyllata*; Myrtaceae; partial hydrolysis; tannase

As a part of chemical investigation on tannins from crude drugs, we have examined the tannins in colves, the dried flower buds of *Eugenia caryophyllata* Thunb. (*Syzygium aromatica* Merr et Perry, Myrtaceae), and isolated a new tannin named eugeniin, together with ellagic acid and gallic acid. This paper deals with the structure determination of eugeniin.

Eugeniin (I), a light tan amorphous powder, $[\alpha]_D$ +57.1° (acetone), is very soluble in water, MeOH, acetone and EtOAc, and shows a blue color with $FeCl_3$ reagent and dark green coloration (after 30 min) with AcOH–$NaNO_2$ reagent,[1] being characteristic of ellagitannin.

1) E.C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).

Table I. PMR Spectra[a] (δ Values)

	Glucose							Galloyl	HHDP
	C_1–H	C_2–H	C_3–H	C_4–H	C_5–H	C_6–H_1	C_6–H_2		
I	6.21 (d, J= 8 Hz)	5.59 (t, J= 8 Hz)	5.85 (t, J= 9 Hz)	5.42 (t, J= 10 Hz)	4.53 (d.d, J= 6, 10 Hz)	5.38 (d.d, J= 6, 14 Hz)	3.88 (d, J= 14 Hz)	6.96, 7.00, 7.12	6.48, 6.65
III	4.5—4.9			5.12 (m)	4.35 (m)	5.18 (m)	3.5—4.0 (m)	—	6.59, 6.68
IV	4.91 (brd, J= 10 Hz)	5.50 (t, J= 10 Hz)	4.70 (t, J= 8 Hz)	5.20 (m)	4.56 (m)	5.28 (m)	3.5—4.0 (m)	7.01	6.43, 6.63

a) Measured in acetone-d_6 at 100 MHz with TMS as an internal standard.
d: doublet, brd: broad doublet, d.d: double doublets, t: triplet, m: multiplet.

Hydrolysis of I with 4 N H_2SO_4 afforded gallic acid, ellagic acid and glucose. The proton magnetic resonance (PMR) spectrum of I exhibits the presence of three galloyl (δ 6.96, 7.00, 7.11 ppm) and one hexahydroxydiphenoyl (HHDP) groups (δ 6.48, 6.63 ppm) in addition to seven well-resolved glucose protons (Table I).

Methylation of I with dimethyl sulfate and K_2CO_3 in dry acetone yielded a crystalline pentadeca-O-methyl ether (II), $C_{56}H_{60}O_{26}$, mp 133—135°, $[\alpha]_D$ +12.6° (acetone), which shows molecular ion at *m/e* 1148 on the mass spectrum (MS).

Based on the above evidences, I requires the structure in which three galloyl and one HHDP groups were attached to glucose nucleus through ester linkage.

In order to elucidate the position of galloyl and HHDP groups on glucose unit, partial hydrolysis with tannase in 0.02 M acetate buffer (pH 5.0) was attempted to give two pale brown amorphous hydrolysates, (III), $[\alpha]_D$ +45.0° (acetone), and (IV), $[\alpha]_D$ +52.1° (acetone). The PMR spectrum of III, as shown in Table I, suggests that all of three galloyl groups were hydrolyzed, while HHDP group was still intact, and since absence of C_1-,C_2-,C_3-protons were observed in the lower field (δ 5.3—6.3 ppm), HHDP group is probably combined with 4,6-position on glucose molecule. Hydrolysate (IV), on the other hand, still contains one galloyl and HHDP groups whose positions were deduced respectively to be 2 and 4,6 on glucose molecule by comparison of PMR chemical shifts with those of I. Methylation of III with dimethyl sulfate and K_2CO_3 in dry acetone, followed by Kuhn methylation,[2] afforded two kinds of permethylates, α-form (V) and β-form (VI), which show closely similar fragmentation pattern with

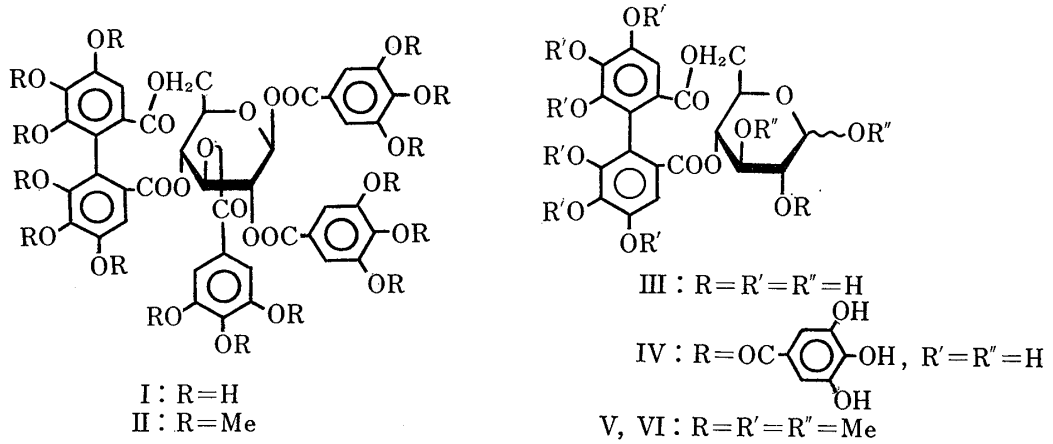

Fig. 1

2) R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, **88**, 1492, 1960 (1955).

each other on MS, having the same molecular ion at *m/e* 608. Both were separately subjected to methanolyses to yield same methyl sugars which were characterized to be methyl 2,3-di-O-methyl β-(α-)glucopyranosides by gas–liquid chromatography.

The configuration of anomeric center on glucose in I was determined to be β-form on the basis of coupling constant (J=8 Hz) on the PMR spectrum of I.

Thus, the structure of eugeniin is established to be 1,2,3-trigalloyl 4,6-hexahydroxydiphenoyl β-D-glucopyranose.

Acknowledgement We are grateful to Professor T. Kawasaki of Kyushu University and to Dr. Okazaki of Sankyo Co., Ltd. for generous supply of methylated sugars and tannase, respectively. We are also indebted to the staff of central analysis room of this faculty for PMR and mass spectral measurements.

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Received December 15, 1979