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Trapain, a New Hydrolyzable Tannin from *Trapa Japonica* Flerov.

A novel hydrolyzable tannin named trapain has been isolated from *Trapa japonica* Flerov. (Oenotheraceae), and the structure has been established as II on the basis of the spectroscopic and chemical evidences.

Keywords——trapain; hydrolyzable tannin; *Trapa japonica*; Oenotheraceae; reduction of dehydrohexahydroxydiphenoyl group; eugeniin; absolute stereostructure

The recent investigation on the tannins in fruit shells of *Trapa japonica* Flerov. (Oenotheraceae) revealed the presence of an ellagitannin, eugeniin (I), and gallotannins such as 1,2,6-trigalloyl, 1,2,3,6-tetragalloyl and 1,2,3-trigalloyl-6-digalloyl glucoses,[1] We now report that further examination of the tannin fraction has resulted in the isolation and characterization of a novel hydrolyzable tannin named trapain.

The ethyl acetate soluble portion of the aqueous acetone extract was repeatedly chromatographed over Sephadex LH-20 (solvent: EtOH, 80% aq. MeOH) to yield yellow crystalline trapain (II), $C_{41}H_{30}O_{27}\cdot 2H_2O$, mp 248—250°, $[\alpha]_D$ +106.2°. The proton nuclear magnetic resonance (PMR) spectrum of II exhibits the signals of three galloyl groups (δ 7.06, 7.12, 7.16), two aromatic and/or olefinic protons (δ 6.41, 6.83) and fully acylated glucose protons which are closely related with those of I,[2] besides a sharp singlet signal (δ 4.66) of a methine proton (Table I). The existence of five ester groups (δ 164.1, 164.8, 166.0, 166.3, 168.2) is confirmed by the carbon-13 nuclear magnetic resonance (CMR) spectrum of II, which also shows the presence of a conjugated carbonyl group (δ 118.9, 131.0, 191.8) and two hemiacetal and/or *gem*-diol carbons (δ 91.1, 96.4), suggesting that II has a hydrated cyclohexenetrione (HCHT) moiety like geraniin,[3] terchebin[4] and mallotusinic acid.[3]

Reaction of II with *o*-phenylenediamine in 15% acetic acid solution afforded a phenazine derivative (III), a yellow amorphous powder, $C_{47}H_{32}O_{24}N_2\cdot 2H_2O$, $[\alpha]_D$ +188.4°, which reveals on the PMR spectrum a sharp singlet (δ 7.89) and four proton multiplet signals (δ 7.84—8.32) attributable to the phenazine moiety.

Reduction of the carbonyl group and subsequent aromatization of the HCHT moiety in II was achieved in high yield by the reaction with 10% aq. $Na_2S_2O_4$ to afford an off-white amorphous powder which was identified as I by the optical rotation and the PMR spectrum,

Table I. PMR Spectral Data of I, II and III (δ-Values)[a,b]

	Glucose							Galloyl	$C_{1'}$–H	$C_{3'}$–H	$C_{3''}$–H	Phenazine moiety
	C_1–H	C_2–H	C_3–H	C_4–H	C_5–H	C_6–H_1	C_6–H_2					
II	6.36 (d, J=8)	5.76 (t, J=8)	6.02 (t, J=9)	5.80 (t, J=10)	4.40 (d.d, J= 6, 10)	5.16 (d.d, J= 6, 10)	4.18 (d, J=10)	7.06, 7.12 7.16	4.66	6.41	6.83	—
I	6.21 (d, J=8)	5.59 (t, J=8)	5.85 (t, J=9)	5.42 (t, J=10)	4.53 (d.d, J= 6, 10)	5.38 (d.d, J= 6, 14)	3.88 (d, J=14)	6.96, 7.00, 7.12	—	6.48	6.65	—
III	6.26 (d, J=8)	5.62 (t, J=8)	5.93 (t, J=9)	5.36 (t, J=10)	4.65 (d.d, J= 6, 10)	5.42 (d.d, J= 6, 14)	4.14 (d, J=14)	6.96, 6.99, 7.12	—	7.89	6.71	7.84—8.32 (m)

a) Measured in acetone-d_6 at 100 MHz with TMS as an internal standard.
d: doublet, d.d: double doublets, t: triplet, m: multiplet.
b) J-values are expressed by Hz.

TABLE II. CMR Spectral Data of I, II and III (δ-Values)[a]

		II	I	III
Glucose	C_1	92.6	93.1	93.5
	C_2	70.9	71.3	71.8
	C_3	72.7	72.3	72.8
	C_4	68.9	70.2	70.8
	C_5	73.2	72.8	72.8
	C_6	65.7	63.1	64.3
Galloyl	C_1	119.2	118.4	119.3
		119.4	118.9	119.9
		119.6	119.0	120.0
	C_2	109.9(6C)	109.6(6C)	109.9(4C)
				110.0(2C)
	C_3	145.7(6C)	145.0(2C)[b]	145.5(2C)
			145.2(2C)[b]	145.7(2C)
			145.3(2C)[b]	145.8(2C)
	C_4	139.3(2C)	138.9	139.0
		139.7	139.0	139.2
			139.5	139.7
-COO-		164.1	164.8	164.6
		164.8	165.6	165.4
		166.0	166.3	165.9
		166.3	167.3	167.2
		168.2	168.1	167.5
DHHDP HHDP	$C_{1'}$	42.7	115.1[c]	114.9
	$C_{2'}$	118.9	124.7[d]	119.2
	$C_{3'}$	131.0	107.5	118.5
	$C_{4'}$	191.8	143.8[b]	145.5[f]
	$C_{5'}$	96.4	135.7[e]	137.0
	$C_{6'}$	91.1	144.6[b]	144.5[f]
	$C_{1''}$	112.3	115.3[c]	116.1
	$C_{2''}$	122.8	125.2[d]	123.7
	$C_{3''}$	107.6	107.5	107.8
	$C_{4''}$	141.9	144.6[b]	145.0[f]
	$C_{5''}$	135.5	136.0[e]	135.8
	$C_{6''}$	150.8	144.6[b]	151.3
Phenazine moiety				130.0(2C)
				132.0(2C)
				142.4, 142.6

a) Measured in acetone-d_6+D_2O at 25.05 MHz with TMS as an internal standard.
b, c, d, e, f) Assignments may be interchangeable.

thus indicating that the dehydrohexahydroxydiphenoyl (DHHDP) group is combined to the C_4- and C_6-hydroxyl groups in glucose moiety.

The orientations of the DHHDP group in C_4- and C_6-positions in glucose moiety was deduced to be C_6: an HHDP part and C_4: a galloyl part, based on the facts that the signals of the C_4–H in glucose moiety on the PMR spectra of I and III are shifted to the upper field (I: δ 5.42; III: 5.36) as compared with that of II (δ 5.80), due to the anisotropic effect of the aromatic ring (Fig. 2). In addition, these upfield shifts of C_4–H in glucose moiety imply not only that the atropisomerism of the HHDP group in I is *S*-series, but also the $C_{1'}$-atom of the HCHT moiety in II has *S*-configuration. Anticipated dimethyl 4,5,6,4′,5′,6′-hexamethoxydiphenoate[3,5] ($[\alpha]_D$ −31.3°, acetone) having *S*-configuration was obtained from the pentadeca-O-methyl ether of I by alkaline hydrolysis followed by methylation with diazomethane, thus concluding the absolute configuration of the C_1'-atom in the HCHT moiety to be *S*.

Unlike the compounds having the DHHDP group such as geraniin, terchebin and mallotusinic acid, trapain does not form an equilibrium mixture in acetone containing water.

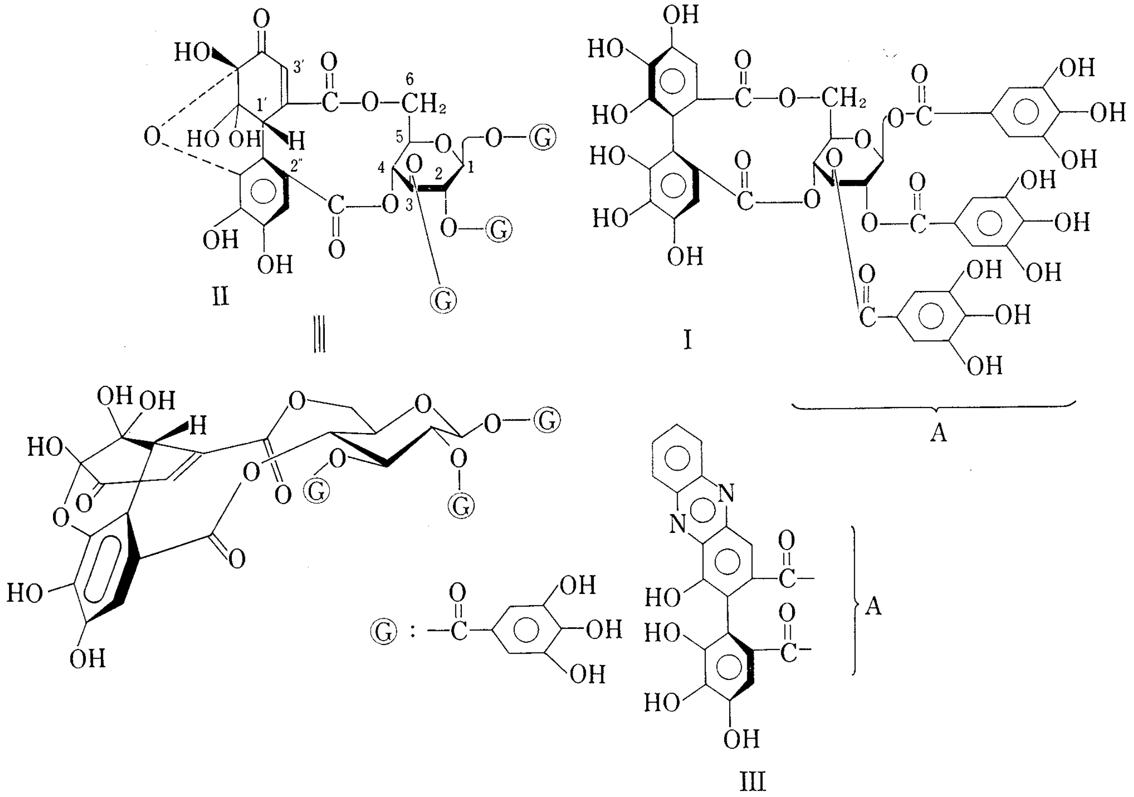
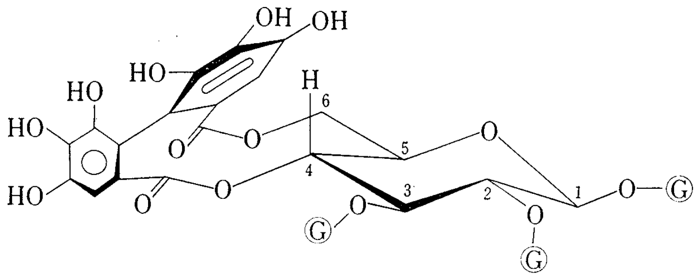


Chart 1

The presence of a rigid six-membered hemiacetal ring in the DHHDP moiety is confirmed on the CMR spectrum of II by the long range selective proton decoupling technique ($C_{1'}$-, $C_{3'}$–H and $C_{5'}$, $C_{6'}$) which establishes the assignments of a hemiacetal (δ 96.4) and a *gem*-diol (δ 91.1) carbons.[4a)]



I

Chart 2

References and Notes

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Synthesis of 25-Hydroxy-24-oxovitamin D_3, A New Metabolite of Vitamin D_3

The synthesis of 25-hydroxy-24-oxovitamin D_3 is described. The compound is identical in all respects with the natural product.

Keywords——vitamin D metabolite; synthesis; Moffatt oxidation; α-Sulphonylcarbanion; epoxy alcohol; steroid

There has been considerable debate, in recent years, regarding the function of 24*R*,25-dihydroxyvitamin D_3,[1] one of the major metabolites of vitamin D_3.[2] Recently, we have isolated and identified a new metabolite of vitamin D_3, 25-hydroxy-24-oxovitamin D_3 (**1**), from chick kidney homogenate incubated with 25-hydroxyvitamin D_3 and suggested the metabolite to be derived from 24*R*,25-dihydroxyvitamin D_3.[3] In order to confirm the structure and to test the biological activity in detail, we carried out the synthesis of 25-hydroxy-24-oxovitamin D_3 and now report the first synthesis of the new metabolite (**1**).

The skeleton of the title compound was constructed from the C-22 steroid sulphone (**2**) which was obtained from ergosterol *via* the established route[4] and the epoxyalcohol (**3**)[5] prepared from commercially available 3-hydroxy-3-methyl-1-butene by epoxidation. The carbanion derived from the sulphone (**2**) (lithium diisopropylamide, tetrahydrofuran —20°) was reacted with the epoxide (**3**) at —20° to give the triol derivative (**4**) (Y: 85%)

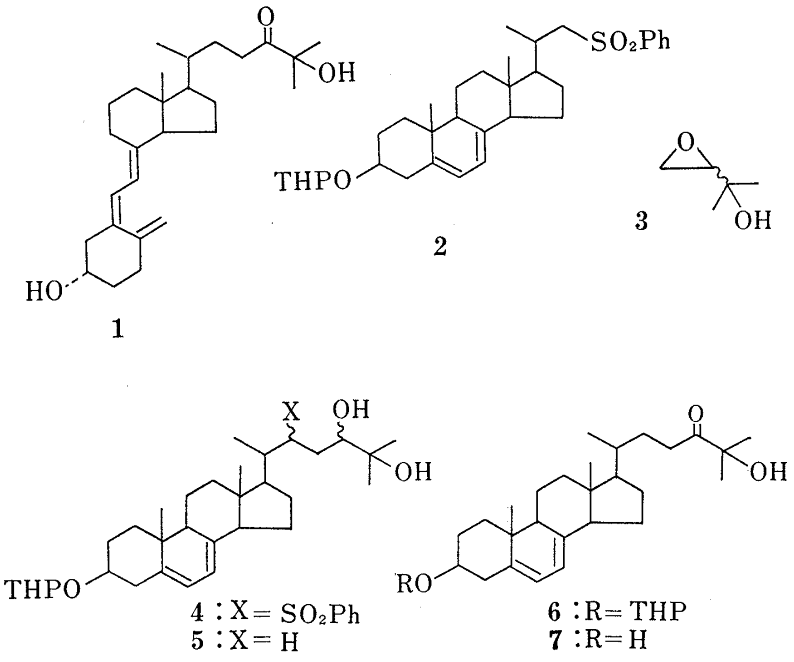

Chart 1