Structure of hippocastanoside, a new saponin from the seed pericarp of horse-chestnut (*Aesculus hippocastanum* L.) I. Structure of the aglycone

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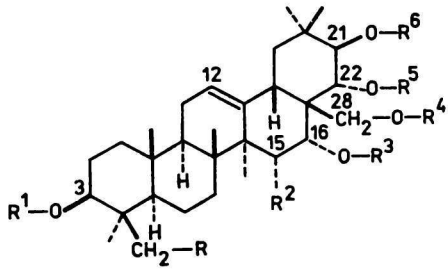
A new saponin designated hippocastanoside was isolated from the seed pericarp of horse-chestnut. The structure of aglycones obtained by acid hydrolysis was elucidated by spectral methods.

Новый сапонин, названный гиппокастанозидом, был выделен из семенной оболочки дикого каштана. Строение агликонов, полученных в результате кислого гидролиза, объяснено с помощью спектральных методов.

Seed constituents of horse-chestnut were investigated completely and in detail [1—3], but little attention has been paid to those of the pericarp. In addition to ubiquitous compounds as saccharides, flavonoids, fats, waxes, the pharmaceutically very important saponins, α- and β-escin were found in seeds [4]. β-Escin is a mixture of triterpenic glycosides the aglycones of which are hydroxy derivatives of olean-12-ene protoescigenin (*I*) and barringtogenol C (*II*); their hydroxyl groups in positions 21β and 22α are esterified with acetic, butyric, 2-methylbutyric, isobutyric, (*Z*)-2-methyl-2-butenoic, and (*E*)-2--methyl-2-butenoic acids. On the other hand, only hydroxycoumarins were identified in the pericarp [5].

The new saponin designated hippocastanoside was obtained from the dry ground pericarp of horse-chestnut seed, from which chloroform-soluble compounds were removed (fats, waxes) by extraction with aqueous ethanol. The extract concentrated under reduced pressure was distributed within a two-phase system consisting of chloroform—2-propanol—aqueous acetic acid; saccharides and procyanidines passed into the upper phase and the saponin, remaining in the lower one, was chromatographically purified and hydrolyzed in acid medium to give a mixture of partially esterified aglycones. Further hydrolysis with alcoholic potassium hydroxide, followed by acidification, work-up and chromatographic separation afforded two compounds (*A*, *B*) in a 1 : 3 mass ratio. Compound *A* ($C_{30}H_{50}O_5$) was acetylated to yield two acetyl derivatives (*C*, *D*) in a 14 : 1 mass ratio.



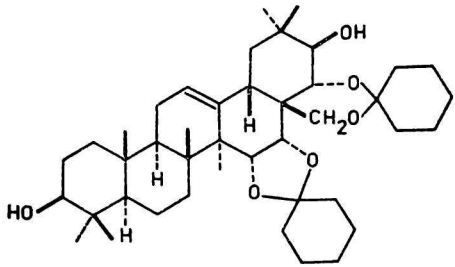
	R	R^1	R^2	R^3	R^4	R^5	R^6
I	OH	H	H	H	H	H	H
II	H	H	H	H	H	H	H
III	H	Ac	H	H	Ac	Ac	Ac
IV	H	Ac	H	Ac	Ac	Ac	Ac
V	H	H	OH	H	H	H	H
VI	H	Ac	OAc	H	Ac	Ac	Ac
VIII	H	H	H	H	H	H	Z
IX	H	H	H	H	H	Z	Z
X	H	H	OH	H	H	H	Z
XI	H	H	OH	H	H	Z	Z

Ac — acetyl; Z — (*Z*)-2-methyl-2-butenoyl.

As proved by the ^{1}H NMR data and elemental analysis, compound *C* gained four and compound *D* five acetyl groups on acetylation thus evidencing the starting product *A* to have all oxygen atoms involved in hydroxyl functions. Compound *A* possessed seven methyl groups attached to quaternary carbons, which means one more methyl group when compared with the structure of protoescigenin (*I*), but only one hydroxymethylene grouping. These data, the melting point and optical rotation value are in line with those reported for barringtogenol C (*II*) [6, 7]. As a consequence, acetyl derivatives *C* and *D* are 3,21,22,28-tetra- (*III*) and 3,16,21,22,28-penta- (*IV*) -*O*-acetylbarringtogenols C [4, 6, 7]. The ^{1}H NMR spectrum of the second sapogenin *B* ($C_{30}H_{50}O_6$), having the same number of methyl and hydroxymethyl groups as barringtogenol C (*II*) differed from *II* only little; small differences exhibited positions of signals attributed to C-3—H, C-12—H, and C-22—H, greater ones C-28—H and C-16—H the latter appearing as a doublet. These data indicate the presence of a further hydroxyl at C-15. Protons C-15—H and C-16—H are in a *cis* arrangement, as follows from the coupling constant $J_{15,16} = 4.3$ Hz. These findings allow to assign saponin *B* the structure of R_1-barrigenol (*V*). On acetylation saponin *B* afforded the pentaacetyl derivative, the ^{1}H NMR spectrum of which

was in a full agreement with that of 3,15,21,22,28-penta-*O*-acetyl-R_1-barrigenol (*VI*) [8, 9].

R_1-Barrigenol (*V*) reacted with two molecules of cyclohexanone to give a compound having its four hydroxyl groups in an acetal grouping. Comparison of the ^{1}H NMR spectrum of this acetal with that of analogous compounds [10—12] allowed to assign it structure *VII*.



VII

The portion of aglycones obtained by acid hydrolysis of hippocastanoside was separated by chromatography to furnish four compounds *E*—*H*. One (*Z*)-2-methyl-2-butenoic acid residue and two the same acyls linked through an ester bond to C-21 and C-22 were found in compounds *E*, *G* and *F*, *H*, respectively. The (*Z*)-isomer of this acid was recognized by position of the signal associated with the vinylic proton in the ^{1}H NMR spectrum at δ/ppm = = 5.90—6.12 (dq); vinylic proton of the more stable (*E*)-isomer was reported [4, 10, 11] to appear at δ/ppm = 6.80—7.00. These findings and preferentially the ^{1}H NMR spectral data (Table 1), together with those published so far entitled to ascribe compounds *E* and *F* structures of 21-*O*-[(*Z*)-2-methyl-2-butenoyl]- (*VIII*) and 21,22-di-*O*-[(*Z*)-2-methyl-2-butenoyl]barringtogenol C (*IX*) and compounds *G* and *H* structures of 21-*O*-[(*Z*)-2-methyl-2-butenoyl]- (*X*) and 21,22-di-*O*-[(*Z*)-2-methyl-2-butenoyl]-R_1-barrigenol (*XI*), respectively.

Ester-bound α,β-unsaturated acids and especially (*Z*)-2-methyl-2-butenoic acid resist acid hydrolysis under conditions required for cleavage of glycosidic bond of the saponin and therefore, aglycones obtained by such a saponification of hippocastanoside do not offer a complete information on their linkage in the native material. Ester bonds involving aliphatic saturated acids or their derivatives undergo cleavage. This hypothesis was verified by an experiment in which hippocastanoside was hydrolyzed with aqueous-methanolic KOH, the solution was concentrated and acidified to pH = 1.5; organic acids were separated by a countercurrent extraction with ether, the extract was dried, concentrated and the remaining acids were converted into methyl esters by reaction with diazo-

Table 1

^{1}H NMR spectral data of compounds *II*—*XI*

Protons	δ/ppm multiplicity[a], J/Hz									
	II	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*
3α-H	3.46	4.50	4.50	3.46	4.53	3.22	3.48	3.27	3.23	3.29
	m	m	m	m	m	dd, 5.3, 10.3	t, 7.9	m	m	m
12-H	5.53	5.37	5.41	5.44	5.48	5.33	5.55	5.46	5.53	5.54
	t, 3.9	t, 3.6	t, 3.4	t, 3.6	t, 3.7	t, 3.5	t, 3.8	t, 3.5	t, 3.6	t, 3.8
15β-H				3.71	4.18	4.40			4.09	3.76
				d, 4.3	d, 4.0	d, 6.8			d, 4.5	d, 4.6
16β-H	4.18	4.18	5.25	4.00	5.10	4.90	4.34	3.95	4.39	3.80
	br. s	br. s	br. s	d, 4.3	d, 4.0	d, 6.8	br. s	br. s	d, 4.5	d, 4.6
21α-H	4.76	5.54	5.35	4.72	5.66	4.14	6.44	5.86	5.47	5.86
	d, 9.8	d, 10.2	d, 10.1	d, 9.6	d, 10.2	d, 10.1	d, 9.8	d, 10.3	d, 10.1	d, 10.3
22β-H	4.55	5.41	5.31	4.57	5.37	3.71	4.78	5.41	3.91	5.41
	d, 9.8	d, 10.2	d, 10.1	d, 9.6	d, 10.2	d, 10.1	d, 9.8	d, 10.3	d, 10.1	d, 10.3
28-H_2	4.08	3.66	3.75	3.59	3.92	3.43	4.03	2.93	3.58	3.00
	d, 11.4	br. s	br. s	dd, 2 × 13.3	d, 11.7	q, 11.7	d, 10.8	br. s	d, 11.5	br. s
	3.77				3.71		3.77	2.89	3.37	2.97
	d, 11.4				d, 11.7		d, 10.8	br. s	d, 11.5	br. s
Me[b]	1.83 s	1.44 s	1.30 s	1.83 s	1.52 s	1.21 s	1.82 s	1.79 s	1.37 s	1.38 s
	1.35 s	1.06 s	1.05 s	1.36 s	1.04 s	1.03 s	1.32 s	1.30 s	1.00 s	1.00 s
	1.32 s	0.96 s	0.96 s	1.31 s	0.98 s	0.98 s	1.23 s	1.23 s	1.00 s	0.99 s
	1.22 s	0.89 s	0.91 s	1.23 s	0.95 s	0.98 s	1.13 s	1.13 s	0.99 s	0.98 s
	1.12 s	0.89 s	0.89 s	1.05 s	0.89 s	0.91 s	1.11 s	1.12 s	0.94 s	0.92 s
	1.05 s	0.88 s	0.87 s	0.98 s	0.87 s	0.88 s	1.06 s	1.01 s	0.89 s	0.89 s
	0.99 s	0.86 s	0.86 s	0.98 s	0.84 s	0.79 s	0.99 s	0.99 s	0.79 s	0.79 s

Table 1 (Continued)

Protons	δ/ppm multiplicity[a], J/Hz							
	II	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*
Ac[b]		2.27 s		2.09 s					
		2.06 s		2.06 s					
		2.05 s		2.05 s					
		2.01 s		2.02 s					
		1.95 s		2.01 s					
21-Z[b]						5.91	6.12	6.09	6.12
						dq, 7.2, 1.5	dq, 7.3, 1.5	dq, 7.3, 1.5	dq, 7.3, 1.5
						2.06	1.96	1.99	1.96
						dq, 7.2, 1.5	dq, 7.3, 1.6	dq, 7.3, 1.5	dq, 7.3, 1.5
						1.99	1.83	1.83	1.83
						dq, 1.5, 1.5	dq, 1.5, 1.6	dq, 1.5, 1.5	dq, 1.5, 1.5
22-Z[b]							6.00		6.02
							dq, 7.2, 1.5		dq, 7.3, 1.5
							1.92		1.93
							dq, 7.2, 1.6		dq, 7.3, 1.5
							1.82		1.82
							dq, 1.5, 1.6		dq, 1.5, 1.5

a) Multiplicity of pea. inglet; d — doublet; t — triplet; q — quartet; br. s — broad singlet.
b) Me — methyl; Ac Z — (*Z*)-2-methyl-2-butenoyl.

methane. The mixture of esters thus obtained was separated by gas chromatography and the particular esters were identified by comparing their retention times with those of the specimens, or alternatively, by combination of gas chromatography and mass spectrometry. Following esters were qualitatively identified: methyl acetate, isobutyrate, butyrate, 2-methylbutyrate, (*Z*)-2--methyl-2-butenoate, (*E*)-2-methyl-2-butenoate, methyl esters of saturated and unsaturated acids of molecular formulas $C_6H_{12}O_2$, and $C_6H_{10}O_2$, $C_7H_{12}O_2$, respectively.

The saponin escin, isolated from the seeds of horse-chestnut contained only acetic, isobutyric, 2-methylbutyric, (*Z*)-2-methyl-2-butenoic, and (*E*)-2-methyl--2-butenoic acids in a 8 : 1 1 : 4 : 6 mole ratio [4, 13], whilst in hippocastanoside the ratio was found to be 7 : 1 : 0.5 : 16 : 2.

Experimental

Melting points were determined on a Kofler micro hot-stage, optical rotation was measured with a Perkin—Elmer, model 141 polarimeter. Gas chromatography was run with a Hewlett—Packard, model 5830 A apparatus using a 10 % Carbowax-20M/Chromaton N-AW-DMCS-packed column (0.100—0.120 mm, 3 m × 2 mm); conditions: 2 min at 50 °C, programmed gradient 7.5 °C min^{-1} up to 140 °C, injection temperature 140 °C, detector temperature 150 °C, carrier gas N_2, flow rate 40 cm^3 min^{-1}. Mass spectra were taken with a Jeol JMS 100 D instrument at an ionization electron energy 70 eV. The ^1H NMR spectra of either CD_3OD, or $CDCl_3$ solutions recorded with a Bruker AM 300 spectrometer operating at 300 MHz are relative to tetramethylsilane. For thin-layer chromatography Silufol sheets (Kavalier, Votice) and solvent systems S_1 chloroform—methanol—water ($\varphi_r = 59 : 33 : 8$), S_2 chloroform—methanol ($\varphi_r = 15 : 2$), and S_3 chloroform—methanol ($\varphi_r = 6 : 1$) were used; detection with vaniline (0.5 g) dissolved in concentrated sulfuric acid (5 cm^3) and ethanol (100 cm^3). The sheets were sprayed prior to heating to 105 °C.

Isolation of hippocastanoside

Dried ground pericarp of horse-chestnut seeds (500 g) was successively extracted with chloroform (2 × 2 dm^3), and ethanol—water ($\varphi_r = 7 : 3$, 2 × 2 dm^3). The filtered aqueous--ethanolic extract was evaporated to dryness, and the residue was dissolved in 2-propanol (200 cm^3). Chloroform (500 cm^3) and dilute acetic acid ($c = 0.5$ mol dm^{-3}, 300 cm^3) were added to the solution, the mixture was lightly shaken and allowed to separate. The lower layer was worked up to give the crude hippocastanoside (3.5 g), which was purified by column chromatography on silica gel. Ethyl acetate—methanol ($\varphi_r = 10 : 1$) removing the coloured substances was substituted by ethyl acetate—methanol—water ($\varphi_r = 9 : 2 : 1$), which eluted hippocastanoside. The composition of eluates was monitored by thin-layer chromatography in S_1. Evaporation of the fraction of $R_f = 0.59$ left

hippocastanoside (1.8 g), m. p. = 228 °C (decomp.), [α] (578 nm, 20 °C, $\varrho = 1$ g dm^{-3}, methanol) = $-21.0°$.

Acid hydrolysis of hippocastanoside

Hippocastanoside (5.0 g) dissolved in methanolic hydrochloric acid (c = = 2.5 mol dm^{-3}, 150 cm^3) was refluxed for 6 h, the mixture was diluted with water (100 cm^3), methanol was evaporated and the residue was extracted with chloroform (3 × 30 cm^3). The combined chloroform extracts were evaporated to dryness and the residue was dissolved in methanol (50 cm^3). Addition of water (30 cm^3) resulted in precipitation of the mixture of aglycones, which was filtered off and purified by column chromatography on silica gel with chloroform—methanol ($\varphi_r = 15:2$). Evaporation of the particular fractions afforded compounds *VIII* (130.8 mg), *IX* (270.1 mg), *X* (224.2 mg), and *XI* (953.9 mg).

21-O-[(Z)-2-Methyl-2-butenoyl]barringtogenol C (VIII), m. p. = 240—242 °C, [α] (578 nm, 20 °C, $\varrho = 0.9$ g dm^{-3}, methanol) = 21.4°, $R_f = 0.46$ (S_2). For $C_{35}H_{56}O_6$ ($M_r = 572.8$) w_i(calc.): 73.39 % C, 9.85 % H; w_i(found): 73.26 % C, 9.72 % H.

21,22-Di-O-[(Z)-2-methyl-2-butenoyl]barringtogenol C (IX), m. p. = 242—243 °C, [α] (578 nm, 20 °C, $\varrho = 1.0$ g dm^{-3}, methanol) = 22.3°, $R_f = 0.67$ (S_2). For $C_{40}H_{62}O_7$ ($M_r = 654.9$) w_i(calc.): 73.36 % C, 9.54 % H; w_i(found): 73.28 % C, 9.41 % H.

21-O-[(Z)-2-Methyl-2-butenoyl]-R_1-barrigenol (X), m. p. = 228—232 °C, [α] (578 nm, 20 °C, $\varrho = 0.9$ g dm^{-3}, methanol) = 16.6°, $R_f = 0.23$ (S_2). For $C_{35}H_{56}O_7$ ($M_r = 588.8$) w_i(calc.): 71.93 % C, 9.59 % H; w_i(found): 71.31 % C, 9.47 % H.

21,22-Di-O-[(Z)-2-methyl-2-butenoyl]-R_1-barrigenol (XI), m. p. = 252—255 °C, [α] (578 nm, 20 °C, $\varrho = 1.1$ g dm^{-3}, methanol) = 16.9°, $R_f = 0.38$ (S_2). For $C_{40}H_{62}O_8$ ($M_r = 670.9$) w_i(calc.): 71.61 % C, 9.32 % H; w_i(found): 71.47 % C, 9.24 % H.

Isolation of sapogenins

Aqueous solution of potassium hydroxide ($c = 2.0$ mol dm^{-3}, 20 cm^3) was added to hippocastanoside (500 mg) dissolved in methanol (100 cm^3). The mixture was refluxed for 1 h, diluted with water (50 cm^3) and methanol was distilled off. The pH of the residue was adjusted with concentrated HCl to pH = 1.5, the separated precipitate (398 mg) was filtered off and the filtrate was countercurrently extracted with ether. Diazomethane dissolved in ether was added to the extract, the solvent was evaporated and the residue was analyzed by gas chromatography/mass spectrometry (Table 2).

Water (10 cm^3) was added to the precipitate dissolved in methanolic HCl ($c = 2.5$ mol dm^{-3}, 40 cm^3) and the mixture was refluxed for 4 h. Methanol was removed and the separated precipitate (185 mg) was filtered off and chromatographed on silica gel. Elution with chloroform—methanol ($\varphi_r = 6:1$) and work-up afforded compounds *II* (28.6 mg) and *V* (90.5 mg).

Barringtogenol C (II), m. p. = 303—304 °C, $[\alpha]$(578 nm, 20 °C, $\varrho = 1.0$ g dm^{-3}, methanol) = 21.8°, $R_f = 0.43$ (S_3). For $C_{30}H_{50}O_5$ ($M_r = 490.7$) w_i(calc.): 73.43 % C, 10.27 % H; w_i(found): 73.38 % C, 10.21 % H.

R_1-Barrigenol (V), m. p. = 299—304 °C, $[\alpha]$(578 nm, 20 °C, $\varrho = 1.0$ g dm^{-3}, methanol) = 28.7°, $R_f = 0.34$ (S_3). For $C_{30}H_{50}O_6$ ($M_r = 506.7$) w_i(calc.): 71.11 % C, 9.95 % H; w_i(found): 71.01 % C, 9.89 % H.

Table 2

Retention times (t) and percentual representation (%) of hydrolyzate components

Peak	Compound	t/min	%
1	Methyl acetate	2.8	13.5
2	Methyl isobutyrate	5.0	4.0
3	Methyl butyrate	6.3	1.1
4	Methyl 2-methylbutyrate	7.1	1.7
5	Methyl (*Z*)-2-methyl-2-butenoate	9.8	52.8
6	Methyl (*E*)-2-methyl-2-butenoate	11.7	7.6
7	$C_5H_{11}COOCH_3$	13.7	2.7
8	$C_5H_9COOCH_3$	15.0	5.1
9	$C_6H_{11}COOCH_3$	15.5	11.5

Acetylation of sapogenins

Barringtogenol C (320 mg) was heated at 90 °C in pyridine—acetic anhydride (15 cm^3 each) for 5 h. The solvents were evaporated and the residue was separated by column chromatography on silica gel using benzene—acetone ($\varphi_r = 5:1$) as eluent. The proper fractions were cleared up and recrystallized from chloroform—heptane ($\varphi_r = 3:1$) to obtain 3,16,21,22,28-penta-*O*-acetylbarringtogenol C (*IV*), yield = 19.1 mg, m. p. = = 149—151 °C, $[\alpha]$(578 nm, 20 °C, $\varrho = 0.9$ g dm^{-3}, chloroform) = −11.5°. For $C_{40}H_{60}O_{10}$ ($M_r = 700.9$) w_i(calc.): 68.55 % C, 8.63 % H; w_i(found): 68.48 % C, 8.54 % H.

Further elution with benzene—acetone ($\varphi_r = 3:1$), work-up and crystallization of the combined fractions from chloroform—heptane ($\varphi_r = 3:2$) yielded 263.6 mg of *3,21,22,28-tetra-O-acetylbarringtogenol C (III)*, m. p. = 223—224 °C, $[\alpha]$(578 nm, 20 °C, $\varrho = 1.0$ g dm^{-3}, chloroform) = 20.2°. For $C_{38}H_{58}O_9$ ($M_r = 658.9$) w_i(calc.): 69.27 % C, 8.87 % H; w_i(found): 69.20 % C, 8.82 % H.

R_1-Barrigenol (150 mg) was acetylated under the same conditions except for the volume ratio of eluent used, which was 3:1. *3,15,21,22,28-Penta-O-acetyl-R_1-barrigenol (VI)*, yield = 126.5 mg, m. p. = 218—220 °C, $[\alpha]$(578 nm, 20 °C, $\varrho = 0.8$ g dm^{-3}, chloroform) = 16.4°. For $C_{40}H_{60}O_{11}$ ($M_r = 716.9$) w_i(calc.): 67.02 % C, 8.44 % H; w_i(found): 66.97 % C, 8.31 % H.

Reaction of R_1-barrigenol with cyclohexanone

R_1-Barrigenol (*V*) (100 mg; 0.2 mmol) and *p*-toluenesulfonic acid (15 mg) were refluxed in a mixture consisting of cyclohexanone (4.7 g; 4.8 mmol) (5 cm^3) and chloroform (30 cm^3) for 3 h, whereby the reaction water was azeotropically removed. The mixture was cooled to ambient temperature, stepwise washed with aqueous solution of sodium hydrogencarbonate ($c = 0.25\ mol\ dm^{-3}$, $2 \times 25\ cm^3$) and water (30 cm^3), the organic layer was dried with sodium sulfate and the solvent was removed by distillation. The residue gave upon crystallization from chloroform—heptane ($\varphi_r = 5:1$) compound *VII* in 77.9 % yield (102.3 mg), m. p. = 221—222 °C, $[\alpha]$(578 nm, 20 °C, $\varrho = 1.0\ g\ dm^{-3}$, chloroform) = 18.7°. For $C_{42}H_{62}O_6$ ($M_r = 665.0$) w_i(calc.): 75.86 % C, 9.70 % H; w_i(found): 75.82 % C, 9.63 % H.

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