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BIOLOGICALLY ACTIVE SAPONINS FROM *DODONAEA VISCOSA*

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Key Word Index *Dodonaea viscosa*; Sapindaceae; saponin esters; R_1-barrigenol; 2,3-dimethyloxiran-2-carboxylic acid; antiexudative activity; phagocytosis-enhancing, molluscicidal activity.

Abstract—From the seeds of *Dodonaea viscosa* a chromatographically pure saponin ester mixture consisting of dodonosides A and B was isolated using DCCC and preparative TLC. The structures of the two compounds were determined by ^{1}H NMR, ^{13}C NMR, FAB MS and GC/MS. Both have R_1-barrigenol as the aglycone and possess an α-L-arabinofuranosyl (1 → 2 or 3)-[β-D-galactopyranosyl (1 → 2 or 3)]-β-D-glucuronopyranose moiety linked to the 3β-hydroxy group. They are esterified at C-21 and C-22 with 2,3-dimethyloxiran-2-carboxylic acid and 2-methylbutyric acid in dodonoside A and 2,3-dimethyloxiran-2-carboxylic acid and angelic acid in dodonoside B. The saponin mixture exerts antiexudative, phagocytosis-enhancing and molluscicidal activity.

INTRODUCTION

Dodonaea viscosa L. Jacq. is widely distributed in tropical and subtropical areas of both hemispheres. It is used in folk medicine as a febrifuge, a diaphoretic drug and also for the treatment of rheumatism and gout [1]. In India, the seeds are used as a fish poison [1]. From the aerial parts of the plant the diterpenoid-derived dodonic [2] and hautriwaic acids [3], some flavonoids [4], tannins [5], sterols [6], plant acids [5] and essential oils [7] were isolated and identified. Apart from a report of the occurrence of saponins in the plant [8], no other chemical work has been carried out to date. We now report on the isolation and structure elucidation of saponin esters from the seeds of *D. viscosa*.

RESULTS

The methanol extract of the seeds was fractionated by butanol extraction and ether precipitation to yield a crude saponin mixture (haemolytic index 30000) which, upon silica gel TLC developed with chloroform–methanol–*n*-propanol–water (9:12:2:8), showed at least six 'Komarowsky positive' zones in the R_f range 0.45–0.55. The main zone had an R_f value of *ca* 0.50. Further separation by DCCC and preparative TLC yielded 30 mg of a chromatographically pure product (main zone), which subsequently turned out to be a mixture of two saponin esters. This mixture could not be separated by any other chromatographic method.

Sapogenin and oligosaccharide part

After alkaline hydrolysis (0.5 M NaOH) of the saponin mixture, a product more polar than the original saponin (TLC) and a mixture of organic acids were obtained and the presence of saponin esters was evident. Alkaline hydrolysis, followed by treatment with hydrochloric acid, yielded a sapogenin, mp 300–305°. The ^{13}C NMR spectrum exhibited signals for 30 carbons and it suggested six hydroxylated (67.46, 67.97, 72.43, 77.41, 78.14, 78.47 ppm) and two unsaturated carbons (124.59, 144.83 ppm). Periodate oxidation indicated vicinal hydroxyl groups. The mass spectrum showed the molecular peak at *m/z* 506.361 ($C_{30}H_{50}O_6$) and the fragmentation pattern of the sapogenin showed its identity with R_1-barrigenol (3β,15α,16α,21β,22α,28-hexahydroxy-12-oleanene), formerly isolated by Hiller *et al.* [9] from *Sanicula europaea*. The identity was proved by co-TLC, IR and a comparison of the physical data with those of an authentic sample.

The oligosaccharide moiety was identified by microhydrolysis on HPTLC, GC analysis of the alditol acetates and by FAB MS of the deacylated saponin as a trisaccharide consisting of arabinose, galactose and glucuronic acid in a molar ratio of 1:1:1. The deacylated saponin was permethylated and then hydrolysed with TFA. The subsequently prepared alditol acetates were subjected to GC/MS analysis in order to establish the sugar sequence and the mode of linkage. Since the partially methylated alditol acetates of 2,3- and 2,4-substituted glucuronic acid give the same mass fragments, we employed $NaBD_4$ instead of $NaBH_4$ for sugar reduction. The identified 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl arabitinol (C1-D), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl galactitol (C1-D) and 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl glucitol (C1-D, C6-2D) revealed an aglycone-bound glucuronopyranose substituted by terminal arabinofuranose and galactopyranose in the 2- and 3-OH positions, respectively. Since we did not succeed in selectively removing one of the terminal sugars it cannot be decided which sugar was attached to the 2- and 3-positions. The arabinose unit has the α-configuration, whereas galactose and glucuronic acid display the β-configuration, as demonstrated by the ^{13}C NMR signals at δ111.03 for arabinofuranose [10–12], and 104.62 and 104.88 for galactopyranose and glucuronopyranose [13–15]. In addition,

the β-linkage of glucuronic acid was confirmed by enzymatic hydrolysis with β-glucuronidase. After permethylation of the deacylsaponin and subsequent TFA hydrolysis we isolated a sapogenin methyl ether which showed the molecular peak at *m/z* 576 and an RDA fragment at *m/z* 368. This fragment corresponded with that of R_1-barrigenol at *m/z* 298, indicating that all the hydroxyl groups with the exception of the 3-OH were methylated. Consequently, the structure of the deacylsaponin was established as R_1-barrigenol-3-*O*-α-L-arabinofuranosyl (1 → 2 or 3)-[β-D-galactopyranosyl (1 → 2 or 3)]-β-D-glucuronopyranoside (**1**).

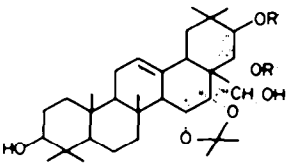
Structure and bonding position of the organic acids

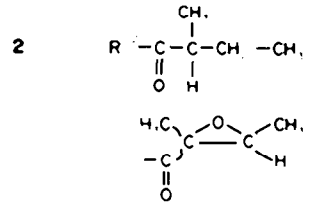
Alkaline hydrolysis of the saponin and extraction with ether yielded two organic acids, which were separated and identified by gas chromatography on a diethylhexylsebacinate-sebacic acid column as methylbutyric acid and angelic acid. The presence of a third non-volatile or unstable acid could be deduced from TLC examination of the ether extract, and the loss of 98 mu in the FAB mass spectrum of the saponin. The finding that the mass difference between the saponin (*m/z* 1157 $[M-H]^-$) and the deacylated saponin (*m/z* 975 $[M-H]^-$) accounted for only 2 mol acids/mol saponin suggested that the acids were distributed on at least two saponin components. This assumption was confirmed by the successful isolation of two genin esters, E-A and E-B, after enzymatic hydrolysis with cellulase from *Aspergillus niger*. Both compounds were converted into monoacetonides by reaction with acetone-*p*-toluenesulphonic acid. Due to the FAB mass spectral fragmentation results, E-A (*m/z* 727 $[M-H]^-$) must be esterified with methylbutyric acid and the third acid of M_r 116 [= 98 + 18(H_2O)], whereas E-B (*m/z* 725 $[M-H]^-$) showed degradation of angelic acid and the third acid. In the ^1H NMR spectra of the monoacetonides of E-A (**2**) and E-B (**3**), this third acid showed a quartet (1H) at δ3.05 (3.03) with a coupling constant of 5.3 Hz, a doublet (3H) with the same coupling constant at 1.35 (1.31) and a singlet integrating for 3H at 1.3. This signal pattern indicates an isolated Me-CH moiety as well as an isolated methyl group. These findings and the M_r of 116 suggested two possible structures; a ketocarboxylic acid (2-methylacetoacetic acid) and an epoxycarboxylic acid (2,3-dimethyloxiran-2-carboxylic acid). The structure of the ketocarboxylic acid could be ruled out because no keto-enol tautomerization upon treatment with $FeCl_3$ reagent and no methyl signal (for the MeCO moiety) in the ^1H NMR spectrum at *ca* δ2.1 were observed. The remaining structure of a 2,3-dimethyloxiran-2-carboxylic acid, hitherto not found in saponins, agreed with the NMR data. In addition, the presence of the epoxy group was demonstrated by a precipitation reaction with a HNO_3–$AgNO_3$–KIO_4 mixture [16]. The methylbutyric acid in E-A was identified as 2-methylbutyric acid due to the methyl signals at δ1.17 (*d*, *J* = 7 Hz) and 0.93 (*t*, *J* = 7 Hz) in the ^1H NMR spectrum [17]. The organic acids in both genin esters must occupy the C-21 and C-22 hydroxyl positions because the protons on C-21 and C-22 in the ^1H NMR spectra of the monoacetonides showed a significant low-field shift in contrast to those of the 15,16-21,22-di-*O*-isopropylidene derivative of R_1-barrigenol (Table 1).

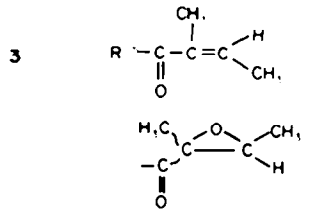
Since, by comparison of the ^1H NMR spectra of both genin esters with and without D_2O exchange, a free $C(28)H_2OH$ group was indicated, the isopropylidene residue must be located at C15,16-OH.

Pharmacological activity

The structural similarity of the *Dodonaea* saponins to antiexudative saponin esters from *Aesculus hippocastanum*, *Thea sinensis*, *Sanicula europaea*, *Eryngium planum*, *Hydrocotyle vulgaris* and *Polemium caeruleum* [18 21] prompted us to subject them to the Viscarin Carrageenin oedema test on a rat paw. At a concentration of 0.7 mg/5 ml/kg an oedema inhibition of







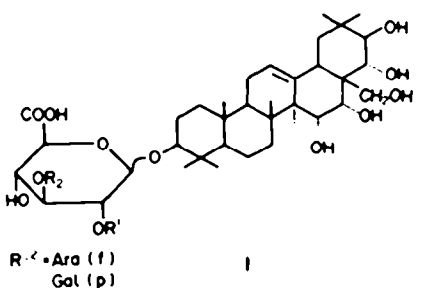


Table 1. ^1H NMR signals of 21β-H and 22α-H of the acetonide derivatives (400 MHz, $CDCl_3$, D_2O exchange)

Acetonide	Chemical shift of 21β-H and 22α-H (ppm)
Diacetonide of R_1-barrigenol	3.70 (*d*, *J* = 10.1 Hz)
	4.11 (*d*, *J* = 10.1 Hz)
Monoacetonide of E-A (**2**)	5.32 (*d*, *J* = 10.1 Hz)
	5.79 (*d*, *J* = 10.1 Hz)
Monoacetonide of E-B (**3**)	5.40 (*d*, *J* = 10.1 Hz)
	5.87 (*d*, *J* = 10.1 Hz)

ca 33% was found, which is about the same order as that observed for aescin. Since according to the investigations of Lutomski [22] some saponins also exert immune-stimulating activities, the *Dodonaea* saponins were also tested in two *in vitro* granulocyte systems, challenged with yeast and zymosan, respectively. In the granulocyte test according to Brandt [23], a dose-dependent enhancement of phagocytosis up to 25% was observed, whereas a phagocytosis-independent increase of luminescence of *ca* 65% was found in the chemoluminescence test according to Allen [24]. Like other saponins, investigated by Hostettmann *et al.* [25] in the molluscicidal test using the bilharziosis-transferring *Biomphalaria glabrata* snails, the *Dodonaea* saponins also showed remarkable activity with 100% lethality at a concentration of 25 ppm.

EXPERIMENTAL

Mps: uncorr; IR: KBr; MS: MS-30 AEI; GC/MS: GC Carlo Erba Fractovap 2110 and MS Varian CH 7A; FAB MS: Kratos MS 50; ^{13}C NMR: Bruker WP 200 (50.3 MHz, C_5D_5N); ^{1}H NMR: Bruker WM 400 (400 MHz, $CDCl_3$, standard TMS); TLC and HPTLC: silica gel 60 F_{254}. For saponins, the lower phase of $CHCl_3$–MeOH–*n*-PrOH–H_2O (9:12:2:8); for monosaccharides, $CHCl_3$–MeOH–H_2O (32:25:5); for sapogenins, CH_2Cl_2–EtOAc–MeOH (10:3:4); for sapogenin esters and acetonides, CH_2Cl_2–EtOAc (13:7); and for acids, *n*-BuOH saturated with 25% NH_3. Spray reagents: for saponins and sapogenins, Komarowsky reagent and citrate blood reagent; for monosaccharides, diphenylamine–H_3PO_4 reagent; and for acids, methyl red indicator, pH 9. DCCC: DCC-A Tokyo Rikakikai, Tokyo, 300 tubes (400 × 2 mm), 15–20 ml/hr, 7–10 kP/cm^2, 12°. GLC: Perkin–Elmer 900, FID, 30 ml/min; columns and column packings: for alditol acetates, GP 3% SP-2330 on 100–120 Supelcoport (225°); for partially methylated alditol acetates, OV 1701 (100°, 3°/min); and for acids, 25% diethylhexyl-sebacinate–sebacic acid on Chromosorb W-NAW (145°).

Plant material. Dodonaea viscosa seeds were provided by the Hamdard Laboratories, New Delhi (India) in autumn 1982. Voucher: No. D-2 Herb. of Inst. of Pharmac. Biology, Munich.

Isolation of the saponins. Powdered seeds (400 g) were treated with petrol in a Soxhlet apparatus for 3 days, dried and extracted with 90% MeOH for 2 days. The MeOH extract was evaporated, dissolved in 1.5 l. H_2O, and shaken with $CHCl_3$ (3 × 1 l.) to remove lipids. The purified H_2O phase was extracted 3 × with 1 l. BuOH saturated with H_2O. The BuOH-soluble fraction was evaporated to yield 20 g of a resinous residue which was dissolved in MeOH and added to 4 l. Et_2O. This procedure was repeated 3 ×. The final residue was dissolved in MeOH. This soln was then treated twice with activated charcoal, filtered, reconcentrated, and poured into Et_2O, to give 11 g of a white saponin mixture. HPTLC: mixture of 6–7 hardly separable Komarowsky-positive spots in the R_f region of 0.50 ($CHCl_3$–MeOH–*n*-PrOH–H_2O, 9:12:2:8, lower phase). Further separation was performed by ascending DCCC with the system used for HPTLC, yielding a mixture of the 'main saponin zone' and one close above. Isolation of the chromatographically pure 'main saponin zone' was achieved by prep. TLC (0.5 mm, 3–4 mg, detection H_2O, eluant MeOH) in $CHCl_3$–MeOH–H_2O (32:25:5); yield: 30 mg; FAB MS: *m/z* 1157 [M – H]$^-$; fragment ions at *m/z* 1073, 1059, 1041, 1025, 995, 977, 959, 941.

Isolation of the sapogenin (R_1-barrigenol). The crude saponin mixture (800 mg) was treated with 0.5 M NaOH for 5 hr at room temp., acidified (pH 1), extracted with BuOH saturated with H_2O, and evaporated to dryness. Acid hydrolysis of the residue (500 mg) with 10% HCl in MeOH for 2 hr under reflux, followed by filtration and washing with H_2O yielded 250 mg of a crude product which was subjected to CC on silica gel (CH_2Cl_2–EtOAc–MeOH, 10:3:4). The main sapogenin, which was also obtained by hydrolysis of the 'main saponin zone', crystallized from $CHCl_3$–MeOH as colourless needles and was identified as R_1-barrigenol by ^{13}C NMR, MS, co-IR and co-TLC. TLC: R_f 0.63 in $CHCl_3$–EtOAc–MeOH (10:3:4), mp 300–305°, $[\alpha]_D^{20}$ + 36° (MeOH; *c* 0.30). IR ν_{max}^{KBr} cm^{-1}: 3360, 2950, 1460, 1380, 1250, 1090, 1030; ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 15.95, 16.57, 17.66, 19.15, 19.35, 21.02, 24.06, 28.24, 28.73, 30.54, 36.35, 36.83, 37.50, 39.38, 39.47, 41.55, 42.12, 47.46, 47.48, 47.88, 48.19, 55.71; (C–OH): 67.46, 67.97, 72.43, 77.41, 78.47; (C=C): 124.59, 144.83.

Periodate oxidation on TLC. The sapogenin was chromatographed (TLC) in CH_2Cl_2–EtOAc–MeOH (10:3:4) together with protoescigenin (Fa. Madaus) as a reference. Spots were visualized by spraying with $NaIO_4$ (0.1% in H_2O) followed by spraying with benzidine 5 min later (1.8 g in EtOH, 50 ml; H_2O, 50 ml; Me_2CO, 20 ml; and 0.2 M HCl, 10 ml; caution, carcinogenic). Both sapogenins appeared as white spots on a blue background.

Microhydrolysis on HPTLC (for sugars). The saponin (main zone) was hydrolysed for 30 min on an HPTLC plate in a HCl chamber at 90°, developed with $CHCl_3$–MeOH–H_2O (32:25:5) and detected with diphenylamine–H_3PO_4 reagent. The hydrolysis products were arabinose, galactose and glucuronolactone (from glucuronic acid).

Alkaline hydrolysis leading to the deacylated saponin. Saponin (main zone) (25 mg) was treated with 0.5 M NaOH for 5 hr at room temp. and acidified with 1 M HCl to pH 1. The deacylated saponin was extracted with BuOH, saturated with H_2O (3 × 5 ml), and evaporated to dryness. FAB MS: *m/z* 975 [M – H]$^-$, main fragment ions at *m/z* 843, 681, 505.

Preparation of the alditol acetates. Deacylated saponin (2 mg) was refluxed for 5 hr with 2 ml 2 N TFA. The soln was evaporated to dryness and the residue dissolved in H_2O, 25 mg $NaBH_4$ was added, the soln was kept at room temp. for 3 hr, acidified with HOAc (2 ml), and evaporated to dryness. The residue was distilled 3 × with MeOH (3 ml) and HOAc (2 ml). The alditols were acetylated with 1 ml Ac_2O for 2 hr at 100°. Ac_2O was removed by co-distillation with toluene. The residue was dissolved in 3 ml $CHCl_3$, washed 3 × with H_2O, and evaporated to dryness. The alditol acetates were dissolved in 20 μl $CHCl_3$ and subjected to GC. The following peaks could be assigned: alditol acetates of arabinose, galactose and glucose (resulting from partially reduced glucuronic acid) in molar ratios of 1:1:0.4.

Permethylation of the deacylated saponin and preparation of the partially methylated alditol acetates. The deacylated saponin (10 mg) was dissolved in DMSO (5 ml) in a 10 ml injection bottle, gased with N_2, and 2.5 ml Na-methyl-sulphinylmethanide was added dropwise. The soln was sonicated for 30 min and then kept at room temp. overnight. The reaction mixture was then chilled in ice and 2.5 ml MeI was added dropwise. The suspension was sonicated for 3 hr, and excess MeI was distilled off. H_2O (5 ml) was added and the permethylated product was extracted with CH_2Cl_2 (3 × 3 ml). The combined CH_2Cl_2 phases were washed 10 × with H_2O and evaporated to dryness. The residue was then hydrolysed with 2 N TFA (5 ml) for 5 hr, and subsequently evaporated to dryness under red. pres. For MS examination, the methylated sapogenin was isolated by prep. TLC (0.5 mm, 4 mg, detection H_2O, eluant CH_2Cl_2) in CH_2Cl_2–EtOAc, 13:7, R_f 0.72. For preparation of the partially methylated alditol acetates, the sample was worked up as described above (preparation of the alditol acetates). Instead of $NaBH_4$, $NaBD_4$ was employed as the reducing agent. By GC/MS analysis of the partially methylated alditol acetates the following main peaks were assigned: 1,4-di-

O-acetyl-2,3,5-tri-*O*-methylpentitol (C1-D): EIMS *m/z* (rel. int.): 43 (100), 44 (13), 45 (39), 71 (13), 87 (21), 101 (20), 102 (28), 118 (100), 129 (54), 161 (25), 162 (5). 1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol (C1-D): EIMS *m/z* (rel. int.): 43 (100), 44 (13), 45 (36), 71 (11), 87 (15), 88 (8), 89 (15), 101 (16), 102 (44), 129 (35), 145 (42), 161 (17), 162 (16), 205 (23). 1,2,3,5,6-Penta-*O*-acetyl-4-*O*-methylhexitol (C1-D, C6-2D): EIMS *m/z* (rel. int.): 43 (100), 44 (24), 86 (12), 89 (17), 100 (9), 127 (22), 131 (66), 160 (4), 191 (34), 202 (5), 262 (15).

Enzymatic hydrolysis with β-glucuronidase. Deacylated saponin (5 mg) was dissolved in 5 ml 0.05 M HOAc NaOAc, pH 5.2, incubated with 5 mg β-glucuronidase from *Helix pomatia* (Sigma Chem. Co., No. G-0751) at 39° for 3 days and extracted 3 × with 2 ml CH_2Cl_2. Evaporation of the combined CH_2Cl_2 phases yielded a residue which was identical with R_1-barrigenol (TLC).

Enzymatic hydrolysis with cellulase. Saponin (main spot) (5 mg) in 5 ml 0.05 M HOAc-NaOAc (pH 5.2) was incubated with cellulase from *Aspergillus niger* (0.3 U/mg, Serva) for 3 days at 39° and the products were extracted with CH_2Cl_2. The extract showed two spots, E-A (R_f 0.5) and E-B (R_f 0.55), in TLC (CH_2Cl_2–EtOAc, 13:7). Larger amounts of the genin esters were obtained by subjecting 1 g of the crude saponin mixture in 500 ml 0.05 M HOAc NaOAc (pH 5.2) to enzymatic hydrolysis with 1 g Cellulase (0.3 U/mg). After 3 days of incubation at 39° the hydrolysate was filtered, the residue washed with H_2O, and dried to give 400 mg of a crude genin ester mixture. CC on silica gel using CH_2Cl_2–EtOAc (13:7) as eluant yielded 55 mg E-A and 20 mg E-B.

Extraction of organic acids. Crude saponin mixture (100 mg) or saponin (5 mg) was treated with 100 ml (5 ml) 0.5 M NaOH at room temp. The soln was acidified to pH 1 with H_2SO_4 and filtered. The filtrate was shaken 3 × with 50 ml (3 ml) Et_2O. The combined Et_2O phases were concentrated and subjected to TLC and GC. (Diethylhexylsebacinate sebacic acid column, 145°, R_t 14.5 min, 12.5 min; spiked with authentic substances).

Acetonide derivatives of E-A (2), E-B (3) and R_1-barrigenol. The sapogenins (5–10 mg) were dissolved in 5–10 ml dry Me_2CO containing 0.5% 2,2-dimethoxypropane. After adding 0.5–1 ml 1% *p*-TsOH in Me_2CO, the solns were gased with N_2 and kept overnight. The reaction mixtures were then treated with 0.1 ml dry pyridine, concentrated under red. pres. to remove Me_2CO, and diluted with ice-water. The colourless ppts. were filtered, washed successively with H_2O, aq. Na_2CO_3, and dried. TLC examination of the products showed that the reaction had been quantitative. Monoacetonide of E-A (2): TLC: R_f 0.75 (CH_2Cl_2–EtOAc, 13:7); ^1H NMR (400 MHz, $CDCl_3$): δ0.91 (3H, *s*, H-23), 0.78 (3H, *s*, H-24), 0.90 (3H, *s*, H-25), 0.87 (3H, *s*, H-26), 1.61 (3H, *s*, H-27), 0.98 (3H, *s*, H-29), 1.05 (3H, *s*, H-30), 1.24 (3H, *s*, acetonide-methyl), 1.47 (3H, *s*, acetonide-methyl), 3.21 (1 H, *m*, 3β-H), 4.18 (1H, *d*, *J* = 6.8 Hz, 15α-H), 3.86 (1H, *d*, *J* = 6.8 Hz, 16α-H), 5.32 and 5.79 (each 1H, *d*, *J* = 10.1 Hz, 21β-H and 22α-H), 2.90 (1H, *d*, *J* = 11.8 Hz, 28-H), 3.28 (1H, *d*, *J* = 11.8 Hz, 28-H), 5.48 (1H, *m*, 12-H). 2-Methylbutyric acid: δ1.17 (3H, *d*, *J* = 7 Hz, α-methyl), 0.93 (3H, *t*, *J* = 7 Hz, β-methyl). 2,3-Dimethyloxiran-2-carboxylic acid: δ3.05 (1H, *q*, *J* = 5.3 Hz), 1.35 (3H, *d*, *J* = 5.3 Hz), 1.3 (3H, *s*). Monoacetonide of E-B (3): TLC: R_f 0.76 (CH_2Cl_2–EtOAc, 13:7); ^1H NMR (400 MHz, $CDCl_3$): δ0.92 (3H, *s*, H-23), 0.79 (3H, *s*, H-24), 0.90 (3H, *s*, H-25), 0.89 (3H, *s*, H-26), 1.61 (3H, *s*, H-27), 0.98 (3H, *s*, H-29), 1.07 (3H, *s*, H-30), 1.25 (3H, *s*, acetonide-methyl) 1.40 (3H, *s*, acetonide-methyl), 3.21 (1H, *m*, 3β-H), 4.19 (1H, *d*, *J* = 6.8 Hz, 15α-H), 3.89 (1H, *d*, *J* = 6.8 Hz, 16α-H), 5.40 and 5.87 (each 1H, *d*, *J* = 10.1 Hz, 21β-H and 22α-H), 2.98 (1H, *d*, *J* = 11.8 Hz, 28-H), 3.30 (1H, *d*, *J* = 11.8 Hz, 28-H), 5.49 (1H, *m*, 12-H). Angelic acid: δ1.85 (3H, *s*, α-methyl), 2.02 (3H, *d*, *J* = 7 Hz, β-methyl), 6.24 (1H, *q*, *J* = 7 Hz, β-H). 2,3-Dimethyloxiran-2-carboxylic acid: δ3.03 (1H, *q*, *J* = 5.3 Hz), 1.31 (3H, *d*, *J* = 5.3 Hz), 1.3 (3H, *s*). Diacetonide of R_1-barrigenol: ^1H NMR (400 MHz, $CDCl_3$): δ0.98 (3H, *s*, 23-H), 0.78 (3H, *s*, 24-H), 0.91 (3H, *s*, 25-H), 0.87 (3H, *s*, 26-H), 1.55 (3H, *s*, 27-H), 0.99 (3H, *s*, 29-H), 1.03 (3H, *s*, 30-H), 1.21, 1.41, 1.46, 1.48 (each 3H, *s*, acetonide-methyl), 3.21 (1H, *m*, 3β-H), 4.39 (1H, *d*, *J* = 6.8 Hz, 15α-H), 4.93 (1H, *d*, *J* = 6.8 Hz, 16α-H), 3.70 and 4.11 (each 1H, *d*, *J* = 10.1 Hz, 21β-H and 22α-H), 3.40 (1H, *d*, *J* = 11.8 Hz, 28-H), 5.32 (1H, *m*, 12-H).

Reagent for epoxide identification. Mixture of 2 ml conc HNO_3, 2 ml $AgNO_3$ in H_2O and 25 ml 2% KIO_4 in H_2O; white ppt.

Bioassays. The antiexudative activity was determined with female Wistar rats. The Viscarin-Carrageenin was injected intraplantar and the saponin mixture was administered i.v. in concns of 0.5, 0.7 and 1 mg/5 ml/kg.

The granulocyte test and the chemoluminiscence test were performed according to Brandt [23] and Allen [24]. Molluscicidal activity was measured with snails of the species *Biomphalaria glabrata* [25]. The haemolytic index was determined according to the methods described in *Europ. Pharmacopoea*.

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