6-Geranyloxy-3-methyl-1,8-dihydroxyanthrone, a novel antileukemic agent from *Psorospermum febrifugum* Sprach var. *ferrugineum* (Hook. fil)

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Summary. A systematic fractionation of the root extract of *Psorospermum febrifugum* was carried out in an attempt to re-isolate the antitumor xanthone psorospermin and related compounds for further structural work and testing. This activity-directed fractionation led to the isolation of 6-geranyloxy-3-methyl-1,8-dihydroxyanthrone (**1**). Compound (**1**) was characterized based on spectroscopic and chemical evidence and showed borderline but reproducible activity in the P-388 mouse leukemia system.

Psorospermum febrifugum Sprach is a woody plant of tropical Africa[2] which is a member of the plant family Clusiaceae (Guttferae)[3]. Several medicinal properties have been ascribed to *P. febrifugum* including its use as a febrifuge, in the treatment of skin ulcerations and in the treatment of leprosy by natives of Angola[2,4].

The ethanol extract of the plant showed activity in P-388 in vivo[5] and 9 KB in vitro[6] and further fractionation led to concentration of the activity in the chloroform layer of a chloroform water partition and in the petroleum ether layer[7] of a 10% aq. methanol and petroleum ether partition. The active petroleum ether fraction was chromatographed on a silica gel column, fractions were combined based on activity against 9 KB and P-388, and the active fraction yielded a light yellow crystalline compound **1** from petroleum-ether acetone[7], m.p. 95–96 °C.

High resolution mass spectrometry established a molecular formula of $C_{25}H_{28}O_4$ (found 392.199, calc. 392.199) for **1** and the ^{13}C NMR-spectrum also indicated a 25-carbon basic skeleton. A geranyl fragment ($C_{10}H_7O$) was clearly disclosed by comparing the NMR of compound **1** with those of geranyl coumarins[9,10]. The ^1H NMR peaks at 2.35 (singlet, 3H) and 4.21 (singlet, 2H) ppm showed the presence of a methyl and methylene groups attached to an aromatic nucleus.

Absorption in the UV at λ_{max}^{MeOH} (log ε) 207 (4.49), 254 (3.89), 270 (3.92), 303 (3.87), 353 (4.14) nm was typical of 1,8-dihydroxy-10-anthrone[8]. The anthrone carbonyl group was further revealed by the ^{13}C signal at 191.3 ppm and IR-absorptions at 1635, 1630 cm^{-1}. The downfield slowly exchangeable proton signals at 12.38 and 12.67 ppm indicated the presence of 2 intramolecularly hydrogen-bonded phenol groups which accounted for the unusual IR-absorption of the carbonyl group at 1630 cm^{-1}. These structural features are also supported by the 4 quarternary ^{13}C resonance signals at 164.9, 164.8, 113.0 and 109.6 ppm.

The location of the hydroxy group was substantiated by the absence of $^{13}C-^1H$ long-range coupling between C_8 and H_1 or H_8. The most upfield aromatic ^{13}C resonance at 99.8 (ddd 161.2, 6.3 and 4.3 Hz) ppm showed its ortho relationship with 2 oxygen functional groups suggesting that the geranyloxy group was attached to the 3 position which was supported by the reduced $^{13}C-^1H$ 3-bond coupling $^3J(C_2-H_4)$ (4.3 Hz)[11]. The meta dioxygen substitution pattern was also in accordance with the upfield 2-proton singlet at 6.41 (H_2 and H_4) ppm. The observation of long-range coupling implied that there was no substituent at the 4 position (C_4 = 106.4 ppm) further suggesting that the methyl group was attached to the A-ring.

The remaining 2 aromatic methine carbon peaks at 119.3 and 115.4 ppm could be attributed to C_5 and C_7 mainly because of the shielding effect of the 8-hydroxy group. This assignment further contributed to locating the methyl group at the 6 position which could explain the absence of $^{13}C-^1H$ 3-bond coupling of the C_6 peak which coupled with the methyl protons only [quartet-$^2J(C_6-C_{11}H_3)$].

This substitution pattern explained the chemical shift difference between C_{1a} (109.4 ppm) and C_{8a} (113.0 ppm) due to the stronger shielding effect of the 3-alkoxy group, and also accounted for $^{13}C-^1H$ coupling patterns of C_{4a} (143.2 ppm; triplet, ^2J ($C_{4a}-H_{10}$): 6.4 Hz), C_{5a} (140.9 ppm; tri-

^1H NMR assignment for compounds **1** and **3**

Carbon	Compound **1**	Compound **3**
H (1)	12.67	12.27
H (2)	6.41c	6.66, d, J = 2.5
H (4)	6.41c	7.3, d, J = 2.5
H (5)	6.67c	7.6, d, J = 1.6
H (7)	6.67c	7.0, d, J = 1.6
H (8)	12.38	12.1
H (10)	4.21	
H (11)	2.35	2.34
H (12)	4.59, d, J = 6.9	4.66, d, J = 6.6
H (13)	5.47, t, J = 6.9	5.54, t, J = 6.6
H (15)	1.75	1.76
H (16)	2.13	2.12
H (17)	2.13	2.12
H (18)	5.17	5.08
H (20)	1.68	1.66
H (21)	1.60	1.60

The NMR-spectrum was recorded on Varian FT-80 NMR-spectrometer utilizing TMS as the initial standard. The NMR-shifts are reported in δ(ppm). J = Hz, c = superimposed singlets, d = doublet, t = triplet.

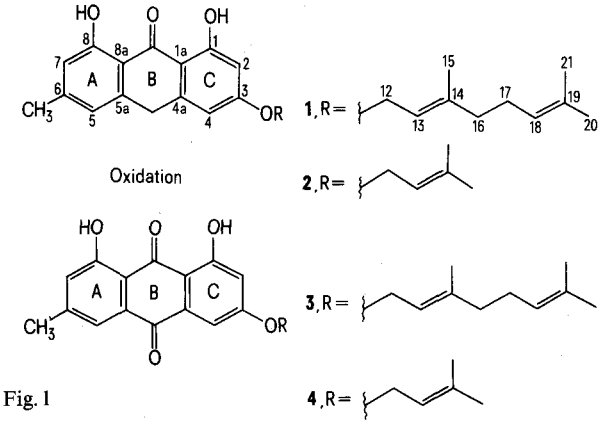

Fig. 1

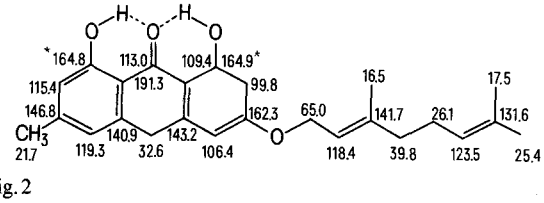

Fig. 2

plet-, ^2J (C_{5a}–H_{10}): 6 · 1 Hz), and C_{10} (32.6 ppm; tt, ^1J (C_{10}–H_{10}): 127.5 Hz, ^3J (C_{10}–H_4) = ^3J (C_{10}–H_5): 3 · 5 Hz). Therefore, compound (**1**) can be assigned as 6-geranyloxy-3-methyl-1,8-dihydroxyanthrone. The basic skeleton and substitution pattern of the aromatic portion is consistent with the polyketide biosynthetic pathway. Furthermore, chemical shifts of the aromatic protons of compound (**1**) are in agreement with those of aromatic protons in the madagascin anthrone[12] (**2**). Oxidation of compound (**1**) in alkaline dimethylsulfoxide[13] gave compound (**3**) which gave UV absorptions at λ_{max}^{MeOH} (log ε), 455 (3.86), 286 (4.07), 262 (4.10), and 221 (4.35) nm. The chemical shifts for the protons in compound (**3**) are comparable to madagascin[12] (**4**).

Efforts to synthesize compounds (**1**) and (**3**) and to establish the important structural features for antitumor activity in this series are presently underway in our laboratory.

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Acidic glycosaminoglycans in human coronary arteries, with special reference to the presence of heparin or related glucosaminoglycan[1]

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Summary. Acidic glycosaminoglycans (AGAG) in 3 branches of the human coronary artery were enzymatically analyzed. The main AGAG were heparan sulfate, chondroitin sulfates and dermatan sulfate. The yield of AGAG decreased in the order of left, right and peripheral branches. Heparin or a related glucosaminoglycan were dominant in the peripheral branch.

Acidic glycosaminoglycans (AGAG) may play a role in the process of atherosclerosis in the coronary arteries as negatively charged macromolecular substances. It is evident that the arterial AGAG possess physiological functions such as antithrombogenic and anti-coagulant properties[2–4]. Numerous studies have reported that the AGAG in the aorta consist of chondroitin sulfates (CS), dermatan sulfate (DS), heparan sulfates (HS) and hyaluronic acid (HA)[3–7]. The presence of a certain amount of heparin in the aorta has been indicated[5,8,9]. In contrast, little is known about the constitution of AGAG in the coronary artery[10,11]. Recently, Likar et al. reported the presence of mast cells in bovine coronary arteries[12]. To the authors' knowledge, no attempt has been made to analyze the AGAG in different branches of the coronary artery.

The present study reports qualitative and quantitative analyses of AGAG in the different branches of the human coronary artery using enzymatic assay[13]. We found a different constitution of the AGAG among the left, right and peripheral coronary arteries. It is of interest to note that heparin or a related glucosaminoglycan are present in human coronary arteries, in the peripheral branch in particular.

Materials and methods. Fresh human coronary arteries were

Composition of acidic glycosaminoglycans in different branches of coronary arteries by enzymatic assay

AGAG	Unsaturated disaccharides	Left (n = 11)[a]	Right (n = 13)	Peripheral (n = 13)
Total AGAG (mg)[b]		5.88 ± 0.43[d]	5.56 ± 0.37	5.12 ± 0.50
Hyaluronic acid (%)[c]	Nonsulfated	5.2 ± 1.2	6.6 ± 0.7	7.1 ± 1.0
Chondroitin 4-sulfate (%)	4-Sulfated	14.6 ± 1.2	11.9 ± 0.7	10.3 ± 0.5
Dermatan sulfate (%)	4-Sulfated	13.2 ± 0.8	15.0 ± 0.8	15.9 ± 1.7
Chondroitin 6-sulfate (%)	6-Sulfated	28.6 ± 1.8	26.4 ± 0.8	26.1 ± 1.9
Oversulfated dermatan sulfate (%)	Di-sulfated	2.8 ± 0.2	3.2 ± 0.6	2.8 ± 0.2
Heparan sulfates (plus heparin) (%)	Undigested with chondroitinase-ABC	35.6 ± 2.2	36.9 ± 2.4	37.8 ± 2.5

[a] Each sample consisted of 3–4 pooled branches based on the closed age in years. [b] Measured as uronic acid and expressed as mg/g defatted dry weight. [c] Measured as uronic acid and expressed as percent to total AGAG. [d] Mean and SE.