Tetrahedron Vol. 45, No. 18, pp. 5907 to 5916, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

NEW TRITERPENES FROM HEINSIA CRINATA

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(*Received in UK* 25 *May* 1989)

Summary : The perchloric acid hydrolysis of a mixture of saponins from the root bark of HEINSIA CRINATA yielded three major genins. Their structures were established on the basis of IR, NMR and Mass Spectrometry. Two of them were shown to be new triterpenes of the cycloartene and lanostene type with a 27-carboxylic group linked to the amino function of 4-hydroxyisoleucine in its γ-lactone form.

INTRODUCTION

Heinsia crinata (Rubiaceae) is a wild plant widely used in folk African medicine against gonorrhoea and diarrhoea. The crude drug is also used as a sexual stimulant.[1,2] Our chemical screening of the root bark of H. crinata indicated the presence of saponins as major constituents of this drug.

This paper describes the isolation of crude saponins and the structure elucidation of three genins obtained by acid hydrolysis.

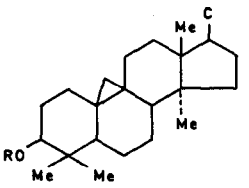
RESULTS AND DISCUSSION

The crude saponins of H. crinata were isolated from methanolic extracts of the root bark using a sequence consisting of extraction with butanol-water and precipitation with isopropyl ether. Perchloric acid hydrolysis of these saponins afforded a mixture of genins. These genins were separated by combined column chromatography and TLC on silica gel to give three crystalline compounds as major sapogenins along with several minor products.

Compound 1 was identified as quinovic acid by comparison of physical and spectroscopic data to those published.[3-6]

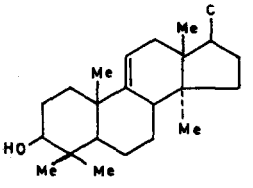
Compound 2a (R=H), m.p.: 166-168°C; $[\alpha]_D$: +138 ($CHCl_3$,c : 1.0), was characterized as the acetate 2b, m.p. : 142°C. On the basis of the arguments set forth below, we attributed to this compound a novel structure, featuring a 9,19-cyclolanostane skeleton and an (E,E)-22,24-dien-27-amide side chain (chart 1). The amino group forms part of a 4-hydroxyisoleucine residue present in the γ-lactone form. In view of the all cis disposition observed for the three ring substituents, the absolute configuration may be assigned as 2S, 3R, 4R, provided that the amino acid belongs to the L-series.

Various carbonyl groups of compound 2b were indicated by the IR spectrum : sec-amide, γ-lactone, acetate ester group (ν_{max}^{KBr} : 3440, 1660, 1510, 1770, 1735, 1245 cm^{-1}). Bands at ν_{max}^{KBr}: 3040, 2960 and 1045 cm^{-1} suggested a cyclopropyl group.[7] The 2,4-dienamide chromophore was revealed by an UV-maximum at $\lambda_{max}(\epsilon)$:252 (3579)nm (solvent : $CHCl_3$).



2a : R=H

2b : R=Ac



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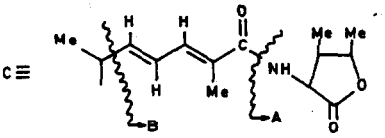


CHART 1 : COMPOUNDS 2 AND 3 AND SOME FRAGMENTATION PATHWAYS OBSERVED IN MS.

The empirical formula $C_{38}H_{57}NO_5$ was established by high resolution mass spectrometry (M^+ measured : 607.4233; theoretical : 607.4236). The most significant fragmentation pathway was provided by allylic cleavage of the 17,20-linkage, allowing a clear differentiation between ions derived from the polycyclic part D and the side chain C. Chart 1 and table 1 indicate the origin and the elemental compositions determined for the more important ions. These assignments were confirmed by exchange with deuterium oxide in the ion source, giving rise to a shift of one mass unit for those ions containing the CONH group, i.e. the molecular ion and the side chain ions B,C and [C + H]. Finally, the two components of the side chain were revealed by secondary loss of the α-aminolactone (AH) from the main side chain ion [C + H], yielding the diene-ketene ion CH_3-CH=CH-CH=CH-$C(CH_3)$=C=O$|^+$ observed at m/z : 122.0735 (base peak).

The ^{1}H-NMR spectrum (Table 2 and fig.1) showed signals corresponding to four tertiary and three secondary methyl groups, one vinylic methyl and one acetyl group. The spectrum also exhibited two proton signals at δ:0.35 (1H, ^{2}J=4Hz) and δ:0.60 ppm (1H, ^{2}J=4Hz) characteristic of the cyclopropyl moiety already indicated by the IR spectrum. These ^{1}H-NMR data (table 2) suggested a 9-19 cyclotriterpenoid.

Decoupling experiments were effected to correlate different signals observed in the spectrum. Irradiation of the doublet at δ : 0.35 ppm changed the doublet at δ : 0.60 ppm to a singlet. On irradiation of the signal at δ:2.23 ppm (1H, m) the methyl signal at δ:1.02 ppm (d, ^{3}J=6.5 Hz) and the vinylic proton signal at δ:5.92 ppm (dd, ^{3}J=9 and 15 Hz) changed into a singlet and a doublet (^{3}J=15 Hz) respectively.

Table 1. The MS data for compounds 2b and 3

2b				3		
m/z	rel.int.(%)	Composition	Fragment	m/z	rel.int.(%)	Fragment
607.4233	7.8	$C_{38}H_{57}O_5N$	M^+	565	51.4	M^+
547.4021	23.2	$C_{36}H_{53}O_3N$	M^+-AcOH	547	8.0	M^+-H_2O
532.3772	15.7	$C_{35}H_{50}O_3N$	M^+-AcOH,-Me	532	11.7	M^+-H_2O,-Me
478.3364	7.2	$C_{32}H_{46}O_3$	M^+-AH	436	9.9	M^+-AH
418.3234	7.2	$C_{30}H_{42}O$	M-AcOH,-AH	-	-	-
357.2755	3.5	$C_{24}H_{37}O_2$	D	315	10.0	D
355.2629	15.1	$C_{24}H_{35}O_2$	D-2H	313	100.0	D-2H
297.2175	30.2	$C_{22}H_{33}$	D-AcOH	297	16.7	$D-H_2O$
295.2425	29.6	$C_{22}H_{31}$	D-AcOH,-2H	295	24.1	$D-H_2O$,-2H
251.1528	69	$C_{14}H_{21}O_3N$	C+H	251	22.1	C+H
250.1438	27.5	$C_{14}H_{20}O_3N$	C	250	20.0	C
222.1137	42.8	$C_{12}H_{16}O_3N$	B	222	46.8	B
178.1672	17.4	$C_{11}H_{16}NO$	$B-CO_2$	178	12.2	$B-CO_2$
122.0735	100.0	$C_8H_{10}O$	CH-AH	122	64.1	CH-AH

Note : A, B, C fragments as formulated in chart 1; D : terpenoid skeleton

Irradiation of the signal at δ:3 ppm (1H, qdd, ^3J=4.5, 7 and 7 Hz) transformed signals at δ : 0.79 (3H, d, ^3J=7Hz), 4.82 (1H, dd : ^3J=5 and 7 Hz) and 4.71 ppm (1H, qd ^3J=4.5, 7 and 7 Hz) respectively into a singlet, a doublet (^3J=5Hz) and a quartet (^3J=7 Hz). On irradiation at δ:4.71 ppm the signals at δ:3 ppm (1H, ddq) and 1.39 ppm (3H, d, ^3J=7Hz) became respectively a double quartet (^3J=4.5 and 7 Hz) and a singlet. On irradiation of the vinylic proton at δ: 6.26 ppm, the doublet of doublet at δ:5.92 ppm became a sharp doublet and the doublet at δ:6.92 ppm became a singlet, broadened by an allylic coupling with the signal at δ:1.98 ppm (d,^4J=1.1 Hz). Upon dilution the doublet at δ:6.30 ppm (^3J=5Hz) shifted upfield, suggesting the presence of a -NH group.

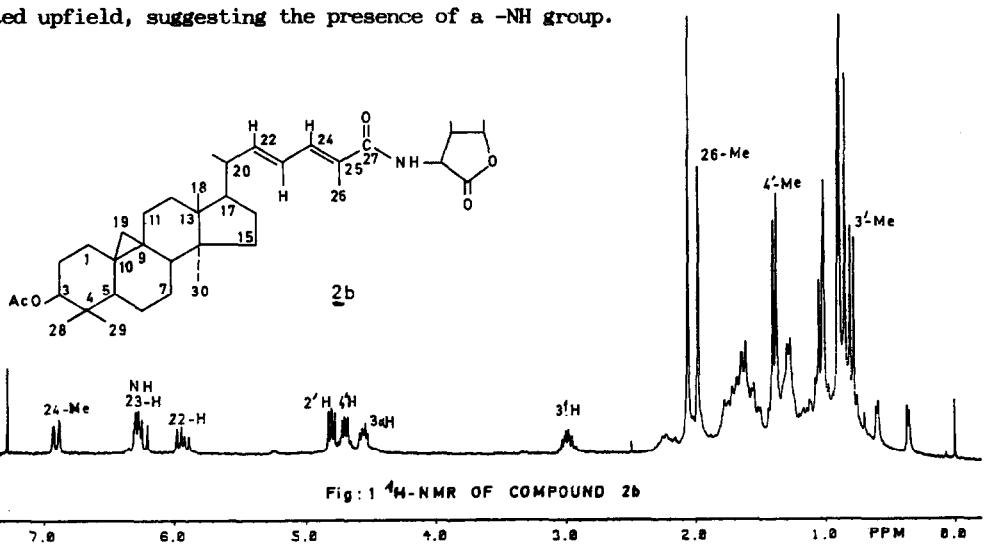
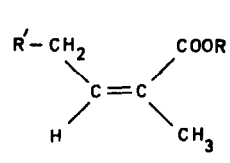


Fig: 1 ^{1}H-NMR OF COMPOUND 2b

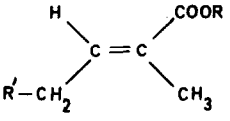
Table 2. Chemical shifts and coupling patterns in the ^{1}H-NMR of 2b and 3

	2b		3	
Groups	δ (ppm)	M : J(Hz)	δ (ppm)	M : J(Hz)
N-H	6.30	d : 5	6.30	d : 5
3α-H	4.57	dd : 5, 11	3.25	dd : 4.5, 11
11-H	-	-	5.25	br.d. : 6
19-CH_2	0.35	d : 4	-	-
	0.60	d : 4	-	-
20-H	2.23	m	2.23	m
22-H	5.92	d.d : 9, 15	5.92	dd : 9, 15
23-H	6.26	dd : 11, 15	6.26	dd : 11, 15
24-H	6.92	d : 11	6.92	d : 11
3β-OAc	2.05	s	-	-
2'-H	4.82	dd : 5, 7	4.82	dd : 5, 7
3'-H	3	qdd : 4.5, 7, 7	3	qdd : 4.5, 7, 7
4'-H	4.71	qd : 4.5, 7	4.71	qd : 4.5, 7
3'-Me	0.79	d : 7	0.79	d : 7
4'-Me	1.39	d : 7	1.39	d : 7
18-Me	0.85	s	0.70	s
19-Me	-	-	1.06	s
21-Me	1.02	d : 6.5	1.06	d : 6.5
26-Me	1.98	d : 1.1	1.98	d : 1.1
28-Me	0.90	s	1.00	s
29-Me	0.88	s	0.85	s
30-Me	1.00	s	0.75	s

The ^{13}C-NMR of compound 2b (table 3) showed 38 carbon atoms : 9 CH_3; 9 CH_2; 6 CH-; 5 C; 2 CH-O-; 3 CH= ; 1 C= and 3 C=O. The presence of the cyclopropyl moiety was confirmed by the signals at δ:20.06 and 26.02 ppm assignable to the quaternary carbons C_9 and C_{10} respectively. The signal for the C_{19} methylene appeared at δ:29.80 ppm. The complete assignment of the carbon signals of the polycyclic part of compound 2b was made by comparison with the published spectra of cyclopropane triterpenoids.[8-10] The assignment of the carbon signals of the side chain (table 3) was based on a series of single frequency selective ^{13}C- ^{1}H decoupling experiments. The spin coupling values in ^{1}H-NMR were used to determine the stereochemistry of the side chain. The large values of the coupling constants between 22-H/23-H (^{3}J=15 Hz) and 23-H/24-H (^{3}J=11 Hz) were consistent with the trans disposition of these protons on the diene. The configuration of the 24-25 double bond was determined by comparison to the literature values shown below.[11]



(Z) $^3J_{COOR,H}$ 13.1 Hz (trans) δ_{CH_3} 20.7 ppm



(E) 7.4 Hz (cis) 11.9 ppm

Table 3. Correlated ^{13}C and ^{1}H chemical shifts [a] of compounds 2b and 3

		2b		3				2b		3	
C	N_H[c]	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	C	N_H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H
1	2	31.6		36.1	1.4-1.8	16[b]	2	28.4		28.3	
2[b]	2	26.8		28.1		17	1	51.8		50.5	1.75
3	1	80.6	4.57	78.8	3.25	18	3	18.2		14.7	0.70
4	0	39.4		39.1		19	2	29.8	0.35-0.6	-	
5	1	47.1		52.5	0.9		3	-	-	22.3	1.06
6	2	20.8		21.3	1.5-1.7	20	1	41.1	2.23	41.1	2.23
7[b]	2	25.8		27.8		21	3	19.5	1.05	19.6	1.06
8	1	47.8		41.8	2.2	22	1	149.1	5.92	149.1	5.92
9	0	20.06		148.6		23	1	122.9	6.26	123	6.26
10	0	26.02		39.4		24	1	135.5	6.92	135.5	6.92
11	2	26.4		-		25	0	126.5		126.5	
	1	-		114.7	5.25	26	3	12.7	1.98	12.8	1.98
12	2	32.8		37	1.9-2.1	27	3	169.4		169.4	
13	0	45.5		44.5		28	3	25.4		28.3	1
14	0	48.9		47.1		29	3	15.1		15.7	0.85
15	2	35.5		33.9	1.35	30	3	19.3		18.5	0.75
MeCO	3	21.3	2.05	-	-	C_3,	1	38.6	3	38.6	3
MeCO	0	170.8		-	-	C_4,	1	77.6	4.71	77.7	4.71
C'_1,	0	175.2		175.3		$\underline{CH_3}$-C_3,	3	7.3	0.79	7.3	0.79
C'_2,	1	55.6	4.82	55.7	4.82	$\underline{CH_3}$-C_4,	3	15.4	1.39	15.5	1.39

(a) $CDCl_3$ as solvent and TMS as reference; (b) alternate assignment is possible; (c) number of protons

The chemical shift observed for 26-Me (12.7 ppm) clearly corresponds to the E configuration. Also the sum of coupling constants ^{2}J and ^{3}J (ΣJ = 17.5 Hz) measured on the ^{1}H-coupled ^{13}C-spectrum between CONH and the protons 26-CH_3, 24-H, -NH, 2'-H, shows that $^{3}J_{CONH,24-H}$ must be considerably lower than the value characteristic for the Z form (±13.1 Hz) . For the γ-lactone moiety, the small values of the vicinal coupling constants ($^{3}J_{H'_2-H'_3}$: 7Hz and $^{3}J_{H'_3-H'_4}$ = 4.5 Hz) were consistent with the cis configuration of the substituents located on the lactone. High values (11.8 and 11 Hz) were observed for the trans configuration.[12,13] The upfield ^{13}C-NMR shifts of the 3'- and 4'-methyl groups (7.25 and 15.4 ppm) also supported the cis configuration. These methyl groups appeared downfield in the trans isomer.[12,13] The free γ-hydroxyisoleucine and the corresponding cis γ-lactone have been reported previously.[14,15]

All these data are in agreement with the structure of a new triterpene of the cycloartene type formulated as N-(2S, 3R, 4R-3-methyl-4-pentanolid-2-yl)-3β-hydroxy-9,19-cyclolanosta-22E,24E-dien-27-amide. It was named heinsiagenin A.

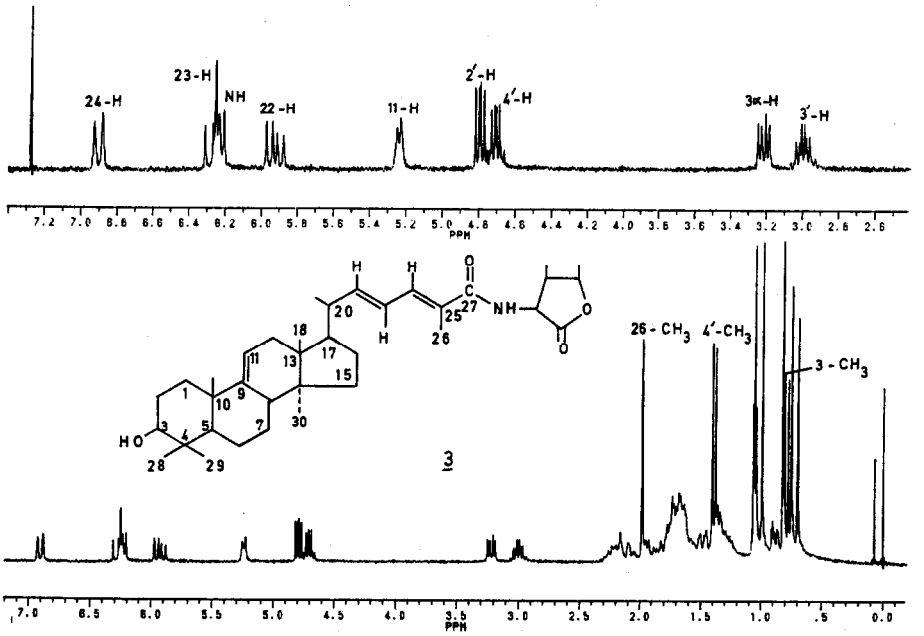


FIG.2: ^{1}H-NMR OF COMPOUND 3

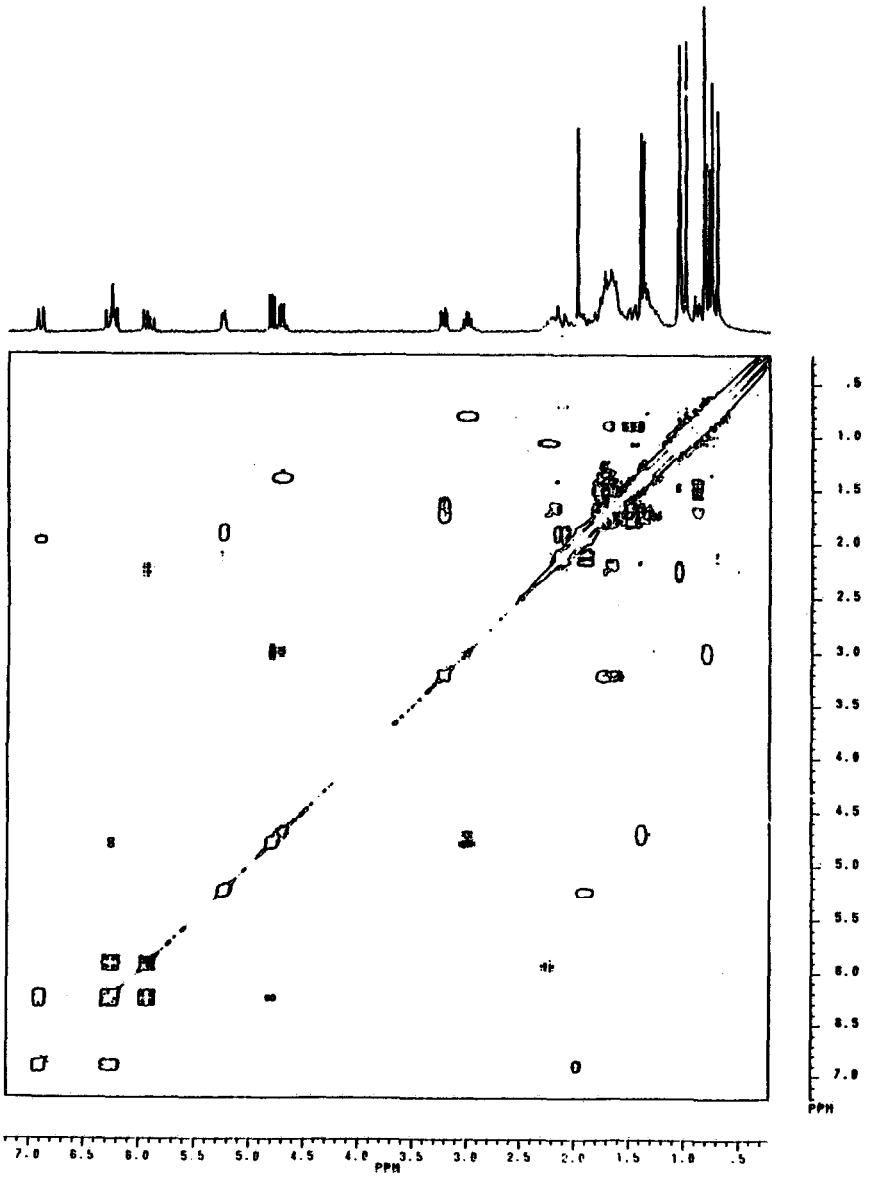
Compound 3 was crystallized from methanol, m.p.: 169-171°C, $[\alpha]_D$: +147.27 ($CHCl_3$, c : 1.1). Its IR spectrum showed bands characteristic of sec-amide and γ-lactone (ν_{max}^{KBr} (cm^{-1}) : 3440, 1660, 1510, 1770 cm^{-1}).

The ^{1}H-NMR spectrum (fig.2) may be referred to that of compound 2b (for comparison, see table 2). Characteristic differences are the disappearance of signals due to the cyclopropyl moiety, the shift of the 3α-H (δ : 3.25 ppm, dd : J=4.5 and 11 Hz) and the appearance of signals due to a sixth tertiary methyl group (δ : 1.06 ppm,s) and a further vinylic proton (δ : 5.25 ppm : $J_{W\frac{1}{2}}$ = 8 Hz). The connectivity of proton resonances for compound 3 was established using 2D-COSY NMR (fig.3).

The ^{13}C-NMR (table 3) showed 36 carbon signals : 9 CH_3, 7 CH_2, 5 CH, 2 CHO-, 4 CH=, 2 C= and 2 C=O. The absence of the cyclopropyl group was confirmed by the lack of characteristic signals. Instead,signals were observed corresponding to the 9-11 double bond[16,17] (δ:114.71 and 148.63 ppm) and to an additional methyl group (19-Me).

The mass spectrum of hydroxy-compound 3 (table 1) was similar to that of acetate 2b suggesting the empirical formula $C_{36}H_{55}NO_4$ (M^+= 565). The base peak at m/z 313 corresponds to the well known [D-2H] fragment ion (loss of side chain with two hydrogen atoms), also observed for compound 2b (m/z = 355) and other triterpenoids.[18] These data indicated that compound 3 is a new triterpenoid of the lanostene type, named N-(2S,3R,4R-3-methyl-4-pentanolid-2-yl)-3β-hydroxylanosta-9(11), 22E, 24E-trien-27-amide (heinsiagenin B).

FIG. 3 : 2D-COSY NMR SPECTRUM OF COMPOUND 3.



EXPERIMENTAL

The plant material was collected in the vicinity of the KOUAMOUTH village (Kimwenza, the region of Kinshasa) and authentified by a voucher specimen H. Breyne 3264 kept in the Herbarium of the INERA, Faculty of Sciences, University of Kinshasa.

Melting points were not corrected. The optical rotations were measured using a polarimeter fitted with a 5 cm cell. IR spectra (KBr pellets) were recorded on a Perkin-Elmer 257 grating IR spectrometer. Mass spectra were run using a Kratos MS50 instrument and a DS90 data system; the ion source temperature was 150-250°C as required. Exact mass measurements were performed at a resolution of 10,000. The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker WM 250 at 250.1 and 62.9 MHz respectively using a 5 mm ^{1}H-^{13}C dual probe. The ^{1}H and ^{13}C chemical shifts are reported in ppm relative to tetramethylsilane as an internal reference. The following solvents were used : DMSO d_6 (^{1}H) and $CDCl_3$-DMSO d_6 (^{13}C) for compound 1; $CDCl_3$ for compounds 2 and 3.

The correlations between ^{13}C and ^{1}H (table 3) were obtained by selective heteronuclear decoupling ^{13}C {^{1}H} in the case of 2b, and by means of a twodimensional heteronuclear correlation experiment in the case of 3.

The DEPT pulse sequence was used to determine the number of protons attached to each carbon. The standard BRUKER microprograms DEPT, COSY and XH CORR were used. Typical acquisition parameters were in the case of DEPT : spectral width 10,000 Hz; pulse angle 45°, 90° and 135°; recycle delay 2 sec; delay time 3.45 msec. COSY-90 : F2 spectral width 2,000 Hz with 1024 data points; F1 spectral width 1,000 Hz with 256 time increments zero filled to 512; 8 scans for each time increment; recycle delay 2 sec, initial delay 3 µsec increment 0.5 msec. XH CORR : F2 spectral width 10,000 Hz with 2048 data points zero filled to 4096; F1 spectral width 1,000 Hz with 256 time increments zero filled to 512; 700 scans for each time increment; recycle delay 1 sec; initial delay 3 µsec, increment 0.25 msec.

Extraction and isolation of saponins

The dried powdered root bark of H. crinata (3.106 kg) was macerated in 80% methanol for 48 hours, the mixture was then refluxed for 3 hours and filtered. The filtrate was evaporated to dryness. The solid residue (530 g) was dissolved in 2600 ml of water and extracted with n-butanol. A triple volume of isopropyl ether was added to the butanolic solution. The precipitated saponins were filtered, washed with isopropyl ether and dried (yield : 201.5 g).

Hydrolysis of saponins and acetylation of genins

The saponins (30 g) dissolved in 100 ml of 3.5 % $HClO_4$ solution were hydrolyzed in a sealed tube at 140°C for 3 hours. The sapogenin precipitate was filtered and washed with water to pH = 7 and dried to yield 13.5 g of sapogenin mixture of which 6 g were acetylated with acetic anhydride (5 ml) and pyridine (5 ml) at room temperature for 48 hours. The acetylated products (6.2 g) were submitted to column chromatography on silica gel (eluent : benzene - ethyl acetate 20 : 1). The fractions containing the major

products were submitted to TLC on silica gel (eluent : benzene - ethyl acetate 9 : 1) to afford pure products 1 (110 mg) and 2b (60 mg). Pure compound 3 (50 mg) was isolated by submitting the non acetylated mixture (3g) of genins to the same separation procedure. Another fraction was proved to be a mixture of naturally acetylated 3 and compound 2b.

A mixture of the crude saponins (100 mg) and β-glucosidase (100 mg), (G0395 Sigma Chemical Company, St. Louis) in acetate buffer (100 ml, pH = 5.5) was incubated at 38°C for 4 days and then extracted with $CHCl_3$. The organic layer was washed with water and concentrated to dryness. The residue (30 mg) was purified by TLC on silica gel, using isopropyl ether acetone 7:3 to afford 20 mg of compound 2a.

Compound 1 crystallized in methanol as small needles; mp : 296-298°C (lit.[6] 282-284); IR : ν_{max}^{KBr} (cm^{-1}); 1735, 1250 (OAc); 1690 (COOH), 1385, 1370 (gem-dimethyl), 1025.

^{1}H-NMR (δ, ppm) 0.78 (d,Me); 0.79 (s,2 Me); 0.81 (d,Me); 0.86 (s,Me); 0.91 (s,Me); 1.98 (s,3β OAc); 4.33 (dd ^{3}J:4 and 12 Hz, 3α.H); 5.50 (t, $w_{\frac{1}{2}}$ = 8Hz, 12-H); 12.1 (br.s 2H 27-COOH, 28-COOH).

^{13}C-NMR : (δ, ppm) 38.1 (C_1); 24.4 (C_2); 80.6 (C_3); 37.45 (C_4), 54.9 (C_5) 18 (C_6); 36.2 (C_7); 39.3 (C_8); 46.4 (C_9); 36.7 (C_{10}); 22.6 (C_{11}); 128.75 (C_{12}); 132.3 (C_{13}); 55.7 (C_{14}); 25.2 (C_{15}); 23.4 (C_{16}); 47.95 (C_{17}); 53.8 (C_{18}); 36.85 (C_{19}); 38.7 (C_{20}); 30(C_{21}); 36.4 (C_{22}); 27.9 (C_{23}); 16.6 (C_{24}); 16,2 (C_{25}); 17.3 (C_{26}); 177.3 (C_{27}); 180.1 (C_{28}); 18.2 (C_{29}); 21 (C_{30}); 20.9 (O-C(=O)-$\underline{C}H_3$) and 170.7 (O-$\underline{C}$(=O)-CH_3).

High resolution ms : m/z (rel.%) : 484.3546 (15) : $C_{31}H_{48}O_4$ (M-CO_2); 469.3306 (51.1) : $C_{30}H_{45}O_4$ (M-CO_2,-Me); 423.3251 (13.8) : $C_{29}H_{43}O_2$; 409.3104 (30.8) : $C_{28}H_{41}O_2$; 363.3041 (14.2) : $C_{27}H_{39}$; 233.1538 (10.5) : $C_{15}H_{21}O_2$; 190.1719 (63.9) : $C_{14}H_{22}$; 189.1648 (41) : $C_{14}H_{21}$; 175.1488 (31.7) : $C_{13}H_{19}$; 135.1179 (42.8) : $C_{10}H_{15}$; 119.0861 (24.2) : C_9H_{11}; 107.0862 (19.2) : C_8H_{11}; 105.0707 (19.4) : C_8H_9.

Compound 2a crystallized from methanol mp : 166-168°C; $[\alpha]_D$: +138 ($CHCl_3$; c : 1.0); ^{1}H-NMR : (δ, ppm) : 0.35 (d, ^{3}J : 4 Hz, 1 H); 0.58 (d, ^{3}J : 4 Hz, 1 H); 0.79 (d, ^{3}J : 7 Hz, Me); 0.81 (s, Me); 0.91 (s, Me); 0.97 (s, Me); 1.02 (s, Me); 1.04 (d, ^{3}J : 6.5 Hz, Me); 1.39 (d, ^{3}J : 7 Hz, Me); 1.98 (d, ^{4}J : 1.1 Hz, Me); 2.24 (m, 1H); 3.00 (qdd, ^{3}J : 4.5,7, and 7 Hz, 1H); 3.29 (dd, ^{3}J : 5, 10 Hz, 1H); 4.70 (qd, ^{3}J : 4.5, 7 Hz, 1H); 4.78 (dd, ^{3}J : 5, 7 Hz, 1H); 5.93 (dd, ^{3}J : 9, 15 Hz, 1H); 6.19 (d, ^{3}J : 5 Hz, 1H); 6.26 (dd, ^{3}J : 11, 15 Hz, 1H) and 6.90 (br.d, ^{3}J : 11 Hz, 1H).

^{13}C-NMR : (δ, ppm) : 7.2; 12.7; 13.9; 15.4; 18.2; 19.3; 19.5 (7 Me); 19.9 (C); 21 (CH_2); 25.4 (Me); 26 (CH_2); 26.1 (C); 26.4; 28.4; 29.9; 30.2; 31.9; 32.8; 35.6 (7 CH_2); 38.5 (CH); 40.4 (C); 41.1 (CH); 45.5 (C); 47; 47.9 (2 CH); 48.9 (C); 51.8; 55.7; 77.7; 78.8 (4 CH); 122.9 (CH); 126.4 (C); 135.7, 149.3 (2 CH), 169.8 (CONH); 175.4 (COO).

For physical and spectroscopic data of compound 2b and 3 we refer to the text and tables 1-3.

Identification of sugars

The aqueous layer obtained after acidic hydrolysis was neutralized with KOH to pH = 7 and filtered. The filtrate was evaporated to dryness, treated with anhydrous pyridine and filtered. Paper chromatography (whatman n°1) of the filtrate (system n - BuOH - Benzene - Pyridine - Water, 5 : 1 : 3 : 3) with authentic monosaccharides as standards revealed the presence of glucose, xylose (scanty) and rhamnose after development with aniline hydrogen phtalate.

Acknowledgements

The authors are indebted to the F.K.F.O. and the "Ministerie voor Wetenschapsbeleid" for financial support and to A.B.O.S. for a fellowship (B.B.). They wish to thank Mr. R. De Boer for technical assistance.

REFERENCES

1. Bouquet, A., *Féticheurs et médecines traditionelles du Congo*, Mémoires Orstom n°36, **1968**, Paris
2. Babady-Bila, *Recherches éthnobotaniques sur les plantes les plus utilisées dans la région de Kinshasa* **1985** (unpublished results)
3. Sahu, N.P.; Mahato, S.B.; Banerji, N.; Chakravasti, R., *Indian J.Chem.* **1974**, 12, 284
4. Adeoye, O.A.; Waigh, R.D., *Phytochemistry* **1983**, 22, 975-978
5. Miana, A.G.; Al-Hazimi, H.M.G., *Phytochemistry* **1987**, 26, 225-227
6. *The Merok Index*, tenth edition, Ed. Merok and Co., Inc., Rahway; **1983**; pp.7998 and references therein
7. Avram, M.; Mateescu, Gh.D.; *Spectroscopie infrarouge, application en chimie organique*, Dunod, Paris; **1970**; pp.240-242, 541-547
8. Radics, L.; Kajtar-Peredy, M.; Corsano, S.; Standoli, L., *Tetrahedron Letters* **1975**, 48, 4287-4290
9. Lakshiminarayama, V.; Murty, Y.L.N., Row, L.R., *Org.Magn.Reson.* **1981**, 17, 77-78
10. Kamisako, W.; Honda, C.; Suwa, K.; Isoi, K., *Magn.Reson.Chem.* **1987**, 25, 683-687
11. Vogeli, U.; von Philipsborn, W., *Org.Magn.Reson.* **1975**, 7, 617-627
12. Raffauf, R.F.; Zennie, Th.M.; Onan, K.D.; Le Quesne, P.W., *J.Org.Chem.* **1984**, 2714-2718
13. Zennie, Th.M.; Cassady, J.M.; Raffauf, R.F., *J.Nat.Prod.* **1986**, 49, 695-698
14. Wieland, Th.; Hasan, M.; Pfaender, P., *Liebigs Ann.Chem.* **1968**, 717, 205-214
15. Fowden, L.; Pratt, H.M.; Smith, A., *Phytochemistry* **1973**, 12, 1707-1711
16. Blunt, J.W.; Munro, M.H.G., *Org.Magn.Reson.* **1980**, 13, 26-27
17. Beierbeck, H.; Saunders, J.K.; Apsimon, J.W., *Can.J.Chem.* **1977**, 55, 2813-2828
18. Enzell, C.R.; Appleton, R.A.; Wahlberg, I. : *Biochemical Applications of Mass Spectrometry*, Ed. G. Waller, Wiley Intersciences, New York, **1972**; pp.351-358