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NEW POLYOXYGENATED STEROIDAL GLUCOSIDES FROM *CHRYSOLINA HYPERICI* (COLEOPTERA : CHRYSOMELIDAE)

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SUMMARY : Two new polyoxygenated steroidal glucosides (1 and 2) have been isolated from the defensive secretion of *C. hyperici* and their structures have been determined by spectroscopic and chemical methods.

Adult chrysomelid beetles belonging to the sub-tribe Chrysolinina are characterized by the production of cardenolides[2-4]. These highly toxic compounds are stored in defensive glands and constitute an efficient protection against predators. However, several Chrysolinina species living on toxic plants are devoid of cardenolides[3]. This is the case of *Chrysolina hyperici* feeding on St John's wort (*Hypericum perforatum*).

This plant contains hypericin, a photodynamic quinone known to be toxic to mammalian herbivores[5]. The defensive secretion of *C. hyperici* does not contain hypericin, but it is characterized by the presence of new polyoxygenated steroidal glucosides. We now report the structure of the two major compounds, 3β-O-β-D-glucopyranosyl-5α-stigmastane-20ξ,25,28ξ-triol-6,16-dione-28-acetate (1) and its corresponding 25-acetate (2). The crude defensive secretion (17 mg), obtained by 'milking'[3] 600 adult beetles, was submitted to repetitive flash silica gel column chromatographies (CH_2Cl_2/CH_3OH 9:1 to 8:2), affording 8.5 mg of 1 and 4.2 mg of 2 (slightly less polar than 1). Compound 1 [$C_{37}H_{60}O_{12}$; amorphous; $[\alpha]_{579}$: -95° (c=0.44), CH_3OH)] shows spectral properties [D/CI MS (NH_3) : 697 (MH^+), 679 (MH^+-H_2O), 661 (MH^+-2H_2O), 484 [$(M+NH_4)^+$+H-side chain], 248 (side chain-H+NH_4^+), 231 (side chain-H+H^+), 213 (side chain-H_2O-H+H^+) ; IR : ν_{OH} 3400 cm^{-1}, $\nu_{C=O}$ 1730 and 1715 cm^{-1}, ν_{C-O} 1245 cm^{-1}; UV : end absorption ^1H NMR : see Table 1] suggesting that it is a polyoxygenated β-glucopyranosyl stigmastane derivative.

The presence of a secondary acetoxyl group at C-28 ($^{29}CH_3$: 1.25 ppm, d(6.5Hz) ; ^{28}CH-OAc : 5.30, dq(6.5 and 3Hz) was proved by irradiation of the dq at δ5.30, which collapsed the $^{29}CH_3$ d at δ1.25 to a singlet, whereas irradiation of the latter simplified the dq to a d (3Hz). The chemical shift and multiplicity of the CH_3 groups at C-21, C-26 and C-27 (see Table 1) strongly suggest the presence of tertiary hydroxyl groups at C-20 and C-25.

Treatment of 1 with $(CH_3CH_2CO)_2O$/pyridine at r.t. for 48h afforded tetrapropionyl-1 (3). The spectral properties of 3 ($C_{49}H_{76}O_{16}$; EIMS : M^+-2H_2O at m/z 884; ν_{OH} 3450cm^{-1}, $\nu_{C=O}$ 1745, 1730 and 1715 cm^{-1} ; ^1H NMR : see Table 1) clearly confirm the above conclusions. Moreover, its ^1H NMR and MS demonstrate that the sugar moiety of 1 is a β-glucopyranose (peaks at m/z 403, 387, 313, 284, 228, 185, 163... 109, characteristic of a tetrapropionyl glucose)[6]. It follows that the steroid aglycone of 1 has the empirical formula $C_{31}H_{50}O_7$, confirmed by HRMS

Table 1 : ^{1}H NMR spectra of compounds 1 to 4 [δ (J in Hz)]

	1+	2*	3≠	4≠
$^{18}CH_3$	0.99 s	0.96 s	0.92 s	0.92 s
$^{19}CH_3$	0.79 s	0.81 s	0.76 s	0.76 s
$^{21}CH_3$	1.29 s	1.29 s	1.26 s	1.26 s
$^{26}CH_3$ [a]	1.22 s	1.48 s	1.20 s	1.46 s
$^{27}CH_3$ [a]	1.22 s	1.54 s	1.25 s	1.51 s
$^{29}CH_3$	1.25 d (6.5)	1.28 d (6.5)	1.27 d (6)	1.25 d (6)
CH_3COO	1.99 s	1.99 s and 2.01 s	2.02 s	1.98 s and 1.99 s
$\underline{CH_3}CH_2COO$ (4)	-	-	1.1, 4 superposed t (7.5)	1.1, 4 superposed t (7.5)
$CH_3\underline{CH_2}COO$ (4)	-	-	2.25, 4 superposed q (7.5)	2.25, 4 superposed q (7.5)
HC-3	3.70 m	3.64 m	3.56 m	3.56 m
HC-5	2.37 m	2.31 m	b	b
HC-28	5.30 dq (6.5, 3)	5.31 dq (6.5, 3)	5.26 dq (6.5, 3)	5.30 dq (6.5, 3)
HC-1'	4.37 d (7.5)	4.41 d (7.5)	4.61 d (7.8)	4.62 d (7.8)
HC-2'	3.12 dd	3.28 dd	4.98 dd, (9.5, 7.8)	4.98 dd, (9.5, 7.8)
HC-3'	c	c	5.20 dd, (9.5, 9.5)	5.20 dd, (9.5, 9.5)
HC-4'	c	3.41 dd	5.10 dd, (9.5, 9.5)	5.10 dd, (9.5, 9.5)
HC-5'	c	c	3.66 ddd (9.5, 4.5, 2.5)	3.65 ddd (9.5, 4.5, 2.5)
H_2C-6'	3.62 dd (12, 5)	3.74 dd (12, 5)	4.24 dd (12, 4.5)	4.24 dd (12, 4.5)
	3.83 dd (12, 2.5)	3.86 dd (12, 3)	4.14 dd (12, 2.5)	4.13 dd (12, 2.5)

a These assignments may be interchanged

b Obscured by other signals

c Obscured by solvent signals

* 250 MHz, $CDCl_3/CD_3OD$, TMS

\+ 400 MHz, CD_3OD, TMS

≠ 270 MHz, $CDCl_3$, TMS

on 1 [m/z 483 (M^+-glucose-H_2O-CH_3) ; $C_{30}H_{43}O_5$)] and on 3 [m/z 481 (M^+-tetrapropionylglucose-H_2O-OH) ; $C_{31}H_{45}O_4$]. The ^{13}C NMR spectrum of 1[7] was fully consistent with these hypotheses and furthermore established that the two remaining oxygen atoms belong to ketone functions (212.5 and 220.4 ppm). These were located at C-6 and C-16 of the steroid skeleton by comparison of the ^{13}C NMR spectrum of 1 with those of 3β-hydroxy-5α-cholestan-6-one[8] and cholest-4-en-20ξ-ol-3,16-dione[9]. The CD of 2 (*vide infra*) is compatible with these hypotheses. The ^1H and ^{13}C NMR data also indicate that the glucose moiety is linked at C-3 of the steroid (HC-3 : 1H multiplet at 3.70 ppm in 1 and at 3.56 ppm in 3 ; ^{13}C NMR : 77.9 ppm ; cholesterol β-D-glucopyranoside[10] : 78.0 ppm).

The spectral properties of 2 [$C_{39}H_{62}O_{13}$; amorphous; $[\alpha]_{579}$: -90.8° (c=0.55, CH_3OH) ; CD : 302 nm, $\Delta\Sigma$-3.8, C_2H_5OH ; D/CI MS (NH_3) : 756 ($M+NH_4^+$), 484 [$(M+NH_4)^+$+H-side chain], 290 (side chain+NH_4^+-H), 212 (side chain +NH_4^+-H-AcOH-H_2O), 180 (glucose) ; ^1H NMR : see table 1] and of its tetrapropionate 4 ($C_{51}H_{78}O_{17}$; EIMS : 884 (M^+-AcOH-H_2O) ; ^1H NMR : see table 1) establish that 2 is the 25-O-acetyl derivative of 1. This was confirmed by mild base treatment of 4 ($NaHCO_3$/MeOH, 15 hrs) affording 2, together with small amounts of 1 and 5, whereas under the same conditions, 3 cleanly affords 5. Treatment of the latter with β-glucosidase afforded 6 [$C_{29}H_{48}O_6$; CIMS (NH_3) : M^+492] the MS of which displays peaks at m/z 139 and 121, characteristic of a 3β-hydroxy-5α-6-ketosteroid[11]. Finally, the presence of a 16-keto-20ξ-hydroxy moiety in 1 and 2 was demonstrated by $SOCl_2$/pyridine dehydration of 7, leading to a mixture of 17,20-double bond isomers, 9 and 10 [MS : 828 (M^+-AcOH) ; IR : $\nu_{C=O}$ 1745 cm^{-1} and 1710 cm^{-1}, $\nu_{C=C}$ 1620 cm^{-1}; UV : λ_{max} 255 nm, ε 8000]. Application of the molecular rotation differences method shows that the sugar is β-D-glucopyranose ($[M]_D$ of 1 : -661° ; $[M]_D$ of 8 + $[M]_D$ of β-D-methyl glucopyranose : -626°). The structure and absolute configuration of 1 and 2 is thus established, except for the configuration at carbon atoms 20, 24 and 25. Biological tests which will be published elsewhere have shown that compound 2 is deterrent for the ant *Myrmica rubra* at concentrations down to 10^{-4}M and toxic at 10^{-3}M.

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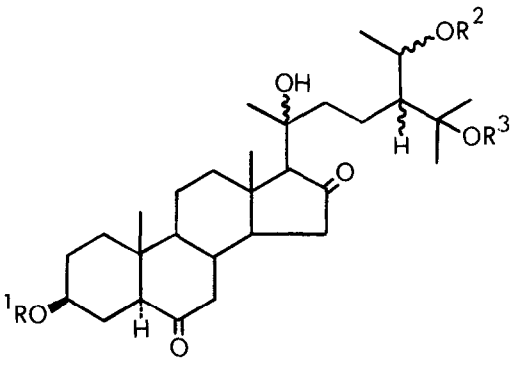
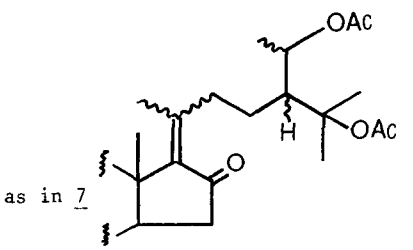
REFERENCES AND NOTES

(1) Maître de Recherches du Fonds National de la Recherche Scientifique.
(2) J.M. PASTEELS, D. DALOZE, Science, 197, 70 (1977).
(3) D. DALOZE, J.M. PASTEELS, J. Chem. Ecol., 5, 63 (1979).
(4) J.M. PASTEELS, D. DALOZE, W. VAN DORSSER, J. ROBA, Comp. Bioch. Physiol., 63C, 117 (1979)
(5) H. BROCKMANN, F. POHL, M.N. HASCHAD, Liebig's Annalen, 553, 1 (1942).
(6) N.K. KOCHETKOV, O.S. CHIZHOV, Adv. Carboh. Chem., 21, 39 (1966).
(7) 100.62 Hz, CD_3OD, δ : C-1, 37.5 ; C-2, 29.9 ; C-3, 77.9 ; C-4, 27.4 ; C-5, 57.6 ; C-6, 212.5 ; C-7, 47.1 ; C-8, 37.5 ; C-9, 54.5* ; C-10, 40.6 ; C-11, 20.5 ; C-12, 40.0 ; C-13, 43.0 ; C-14, 52.0 ; C-15, 42.0 ; C-16, 220.4 ; C-17, 71.9 ; C-18, 13.4≠ ; C-19, 15.2≠ ; C-20, 73.4 ; C-21, 25.9 ; C-22, 44.6 ; C-23, 22.1 ; C-24, 54.6* ; C-25, 75.6 ; C-26, 28.9+ ; C-27, 28.7+ ; C-28, 73.2 ; C-29, 17.3 ; C-1', 102.5 ; C-2', 75.2 ; C-3', 78.2 ; C-4', 72.8 ; C-5', 78.6 ; C-6', 63.0
(+, ≠ and * : these assignments may be reversed).
(8) J.W. BLUNT, J.B. STOTHERS, Org. Magn. Resonance, 9, 439 (1977).
(9) R. BENVEGNU, G. CIMINO, S. de ROSA, S. de STEFANO, Experientia, 38, 1443 (1982).
(10) S. SEO, Y. TOMITA, K. TORI, Y. YOSHIMURA, J. Am. Chem. Soc., 100, 3331, (1978).
(11) F.J. HAMMERSCHMIDT, G. SPITELLER, Tetrahedron, 29, 3995 (1973).



1	R^1 = glucose	$R^2 = COCH_3$	R^3 = H
2	R^1 = glucose	$R^2 = COCH_3$	$R^3 = COCH_3$
3	R^1 = tetrapropionylglucose	$R^2 = COCH_3$	R^3 = H
4	R^1 = tetrapropionylglucose	$R^2 = COCH_3$	$R^3 = COCH_3$
5	R_1 = glucose	R^2 = H	R^3 = H
6	R_1 = H	R^2 = H	R^3 = H
7	R_1 = tetraacetylglucose	$R^2 = COCH_3$	$R^3 = COCH_3$
8	R_1 = H	$R_2 = COCH_3$	R_3 = H



9 and 10

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