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# NEW POLYOXYGENATED STEROID GLYCOSIDES FROM THE DEFENCE GLANDS OF SEVERAL SPECIES OF CHRYSOLININA BEETLES (COLEOPTERA : CHRYSOMELIDAE)

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Abstract. The composition of the defence secretions of four species of *Chrysolina* beetles feeding on St John's wort (*Hypericum perforatum*) has been investigated. In contrast with other members of this genus which produce cardiac glycosides these four species contain new polyoxygenated steroid glycosides. The structure of seven of these derivatives (3-9) have been determined.

Résumé. La composition des sécrétions défensives de quatre espèces de coléoptères du genre *Chrysolina*, inféodées au millepertuis (*Hypericum perforatum*), a été étudiée. Alors que les autres membres de ce genre produisent des glycosides cardiaques, ces quatre espèces contiennent des glycosides stéroïdiques polyoxygénés. Les structures de sept d'entre eux (3-9) ont été déterminées.

## Introduction

Chrysomelid beetles belonging to the sub-tribe Chrysolinina are characterized by the production of cardenolides in defence glands opening at the surface of the pronotum and elytra (1-5). We have shown by incorporation experiments with (4-$^{14}$C)- and (1,2-$^{3}$H;23-$^{14}$C)-cholesterol that these toxic steroids are not sequestered from the host-plants, but that the beetles biosynthesize them from dietary plant sterols (6). Some Chrysolinina species, however, depart from this general trend. *Orcina cacaliae*, for example, does not produce cardenolides but instead contains pyrrolizidine alkaloids probably sequestered from its host-plant (7). In contrast, *Chrysolina hyperici*, exclusively feeding on *Hypericum perforatum* (St John's wort), was found to produce the novel polyoxygenated steroid glucosides 1 and 2 (8).

Several other Chrysolinina species live on *Hypericum* and we now report that three of them, *viz. C. varians, C. brunsvicencis and C. geminata*, are also characterized by the production of closely related polyoxygenated steroid glycosides whose isolation and structure determination are the subject of this paper.

The defence secretions were obtained by milking the beetles as previously described (2). The secretions were stored in methanol and the compounds isolated by silica gel flash chromatography and/or Lobar chromatography (see experimental)

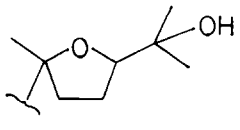
*Chrysolina varians* : The secretion (38 mg from about 2,500 individuals) was analyzed by tlc ($SiO_2$ plates; eluent : $CH_2Cl_2$- $CH_3OH$ 8 :2), showing the presence of two compounds, 3 and 4. After

several flash column chromatographies, pure 3 (18 mg) and 4 (1.5 mg) were obtained.
Compound 3 [($C_{41}H_{66}O_{16}$;$[\alpha]_{579}$ = -36° (c = 0.25, MeOH)], showed spectral properties [(M-H)- at m/z 813 in negative ion FABMS; $^1H$ NMR : table 1; $^{13}C$ NMR : experimental; IR : νOH 3500-3100 $cm^{-1}$; νC=O 1730 and 1705 $cm^{-1}$; νC-O 1245 $cm^{-1}$] suggesting it is a steroid diglycoside bearing a ketone and an acetyl groups. The successive losses of 162 daltons from the $(M+H)^+$ ion in positive ion FABMS together with the NMR data indicated that both sugars are hexoses. The glycosidic moiety was identified to sophorose [β-glucopyrano (1->2) β-glucopyranoside] on the following grounds.

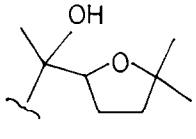
Acid hydrolysis of 3 using Kiliani reagent afforded only glucose, identified by GLC of its TMS derivatives. Furthermore, a $2D^1H/^1H$ NMR spectrum of the corresponding peracetyl derivative (3a) in $C_6D_6$ allowed us to assign all the signals of the osidic part of the molecule (table 2). The 1->2 link between the two glucose units rested upon : i) the comparison of the $^{13}C$ NMR spectrum of 3 with that of β-sophorose (10)[10], ii) the identification of the H-2 signal of the terminal glucose unit at δ 3.75 (dd 9.7, 7.5 Hz) in the $^1H$ NMR of 3a.

The structure of the aglycone was based on spectroscopic data. Indeed, comparison of the $^1H$ and $^{13}C$ NMR spectra of 3 with those of 3β-hydroxy-5α- 6-oxocholestane [11] (to which were added the increments for a 3β-D-glucoside[12]) demonstrated the presence of this structural moiety in 3. The presence of the 5α- 6-oxo steroid system was also supported by CD[13] (287 nm; Δε = -0.78; $CH_3OH$). The acetoxyl function was located at C-16 on the basis of the coupling constants of the 16-CH (δ 5.46; dt 6.7 and 4.2 Hz) and the comparison of the $^{13}C$ NMR of 3 with that of acetyl guggulsterol III[14]. Moreover, the chemical shift of the 18-$CH_3$ (δ 1.06) was also in agreement with this hypothesis[15].

These structural elements amounted to 6 out of the 7 degrees of unsaturation of the molecule. It followed that the side chain must contain the last unsaturation together with the two remaining oxygen atoms. One of them was a tertiary alcohol as shown by treatment of 3a with trichloroacetylisocyanate [16] in $C_6D_6$. The $^1H$ NMR spectrum of the carbamate thus obtained (3b) showed only 1 H below 9.0 (δ 9.79,bs) thus proving that the last oxygen atom is part of an ether bridge.
Examination of the $^1H$ NMR spectrum of 3 led to the conclusion that the two oxygen atoms are located at C-20 and C-25 (21-$CH_3$: δ 1.31; 26-$CH_3$ and 27-$CH_3$ : δ 1.14 and 1.16). Furthermore, the ether bridge must be quaternary-tertiary since only one CH-O signal appears at δ 3.55 in the $^1H$ NMR of 3a. The coupling constants of this proton (6.4 and 7.4 Hz) suggested it is part of a tetrahydrofurane ring. At this stage, only two hypotheses (A and B) fitted the above data for the

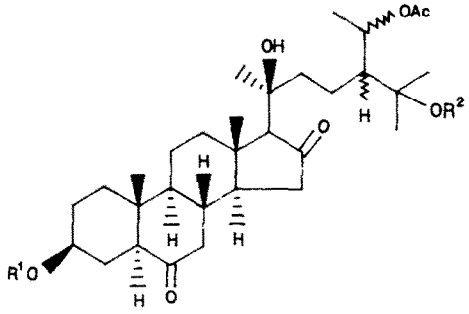



A

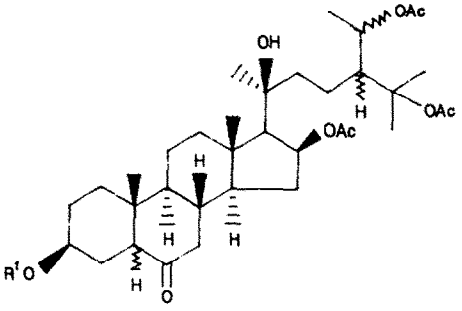



B

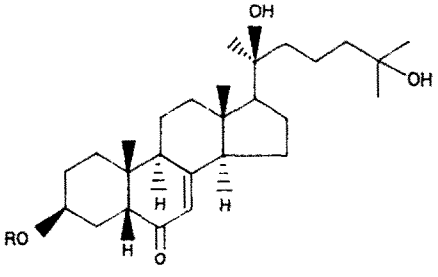
side chain. A choice in favour of hypothesis A could be made on the following grounds : i) the good correlation between the $^{13}C$ NMR values of 3 and those of similar tetrahydrofurane compounds, e.g.



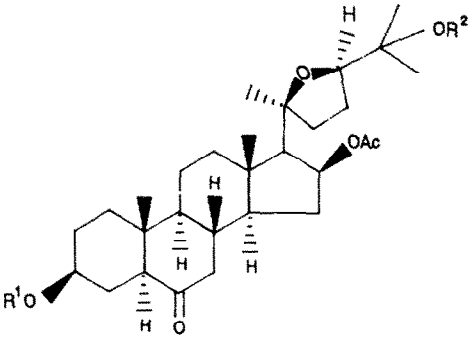
1 $R^1$=β-glucose; $R^2$=H
2 $R^1$=β-glucose; $R^2$=Ac
2a $R^1$=β-acetylglucose; $R^2$=Ac
9 $R^1$=β-sophorose; $R^2$=H
9a $R^1$=β-acetylsophorose; $R^2$=H



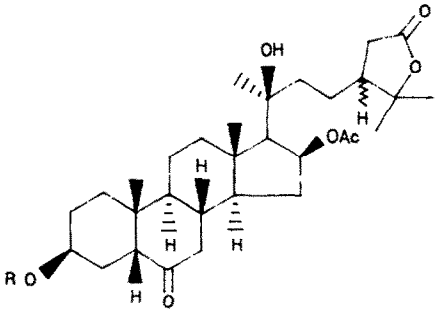
5 $R^1$=β-sophorose; 5βH
5a $R^1$=β-acetylsophorose; 5βH
6 $R^1$=β-glucose; 5βH
6a $R^1$=β-acetylglucose; 5βH
8 $R^1$=β-sophorose; 5αH
8a $R^1$=β-acetylsophorose; 5αH



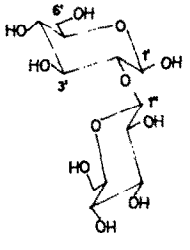
4 R=β-sophorose



3 $R^1$=β-sophorose; $R^2$=H
3a $R^1$=β-acetylsophorose; $R^2$=H
3b $R^1$=β-acetylsophorose;
$R^2$= CO-NH-CO-$CCl_3$



7 R=β-sophorose



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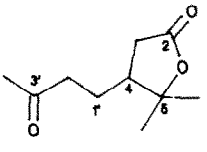

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linalool oxides [17] and dictyol H [18], ii) the loss of 59 daltons from m/z 490 in EIMS, corresponding to cleavage between C-24 and C-25 (m/z 431 : $C_{26}H_{39}O_5$; calc : 431.2799; meas. : 431.2797), iii) the observation of nOe effects between 26-$CH_3$, 27-$CH_3$ and 24-CH, on one hand, and between 24-CH and 21-$CH_3$ on the other hand. This latter result also implied a cis relationship between 24-CH and 21-$CH_3$. Moreover, the "natural" 20(S) absolute configuration may be tentatively proposed for 3, by comparison of its 21-$CH_3$ chemical shift (δ 1.31) with literature values for 20(R)- and 20(S) -3β-acetoxy-20-hydroxy-cholesterol[20] (δ 1.13 and 1.28, resp.). Thus, we propose structure 3 for this compound.

The spectral properties of 4 [$(M-H)^-$ at m/z 755 in negative ion FABMS; UV : λ max 247 nm (ε7,000); IR : ν OH 3500-3100; ν C=O 1635 $cm^{-1}$; $^1H$ NMR : see table 1] suggested the presence of a cholestane derivative bearing a 6-oxo-$\Delta^7$-functionality[19], two tertiary OH groups and a diglycoside moiety. The latter was again identified as β-sophorose by FABMS (peaks at m/z 593 and 431) and $^1H$ NMR spectroscopy, by comparison with the $^1H$ NMR spectrum of 5 (see below and table 1). The location of the two OH groups at C-20 and C-25 relied on the chemical shifts of the 21-$CH_3$, 26-$CH_3$ and 27-$CH_3$ groups (δ 1.28 (3H) and 1.18 (6H) respectively). The 20(S) absolute configuration is again proposed on the basis of the 21-$CH_3$ chemical shift (δ 1.28)[20]. Finally, the A/B cis ring junction, suggested by the chemical shift and the shape of the 3-CH signal (δ 4.1, bs, $w_{1/2}$: 12 Hz), was fully confirmed by decoupling experiments and nOe difference spectra. Indeed, irradiation at δ 2.2 (4-$CH_e$) simplified both the 3-CH signal at δ 4.1 and a dd at δ 2.55, thus assigned to 5-CH. Irradiation of the 19-$CH_3$ signal at δ 0.89 led to nOe effect on 5-CH thus unambiguously showing its 5β-orientation and completing the structure elucidation of 4.

*C. brunsvicencis.* Tlc analyses (same as above) of the secretion (15 mg from 600 individuals) showed the presence of four spots. Repetitive Lobar and flash column chromatographies allowed us to isolate the two major compounds of the secretion, 5 and 6. The spectral properties of compound 5 [$(M - H)^-$ at m/z 943, fragment ion at m/z 781 $(M - H - 162)^-$ in negative ion FABMS, $(M + H)^+$ at m/z 945, fragment ion at m/z 603 $(M + H - H_2O - 2 \times 162)^+$ in positive ion FABMS; IR : νOH 3350 $cm^{-1}$, νC=O 1730 and 1700 $cm^{-1}$, νC-O 1245 $cm^{-1}$; $^1H$ NMR : table 1] suggested it is a steroid dihexoside having an empirical formula $C_{47}H_{76}O_{19}$.

It followed that the aglycone had the formula $C_{35}H_{56}O_9$ corresponding to a stigmastane skeleton bearing three acetoxy groups (δ 1.96, 1.99 and 2.07, s, 3H each). Acetylation of 5 afforded a decaacetate 5a whose $^1H$ NMR spectrum unambiguously showed the presence of an acetylated β-sophorose unit (table 2). The (1->2) link of the sophorose was proved by selective irradiation of 1'-CH at δ 4.44 (d,J = 7.7 Hz) which caused the dd at δ 3.72 (J = 7.7 and 9.3 Hz) to collapse into a doublet (J = 9.3 Hz).

A careful comparison of the $^1H$ NMR of 5 and 5a with those of 2 and 2a[8] showed that the aglycones of these compounds were structurally closely related, the only differences being the replacement of the carbonyl group at C-16 of 2 by an 16β-acetate (16-CH, δ 5.39, J= 4.0 and 6.5 Hz) and the A/B cis ring junction of 5. The latter was indicated, as in 4, by the chemical shifts of the 3-

Table 1: $^1$H N.M.R. data of natural glycosides ($CD_3OD$, TMS, 250 MHz)

| | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|
| 18-$CH_3$ | 1.06 (s) | 0.78 (s) | 1 12 (s) | 1.12 (s) | 1.11 (s) | 1.12 (s) | 1.0 (s) |
| 19-$CH_3$ | 0.77 (s) | 0.89 (s) | 0 87 (s) | 0.86 (s) | 0.87 (s) | 0.77 (s) | 0.80 (s) |
| 21-$CH_3$ | 1.31 (s) | 1.28 (s) | 1 33 (s) | 1.33 (s) | 1.33 (s) | 1.33 (s) | 1.29 (s) |
| 26-$CH_3$ | 1.14 (s) | 1 18 (s) | 1.48 (s) | 1.48 (s) | 1.28 (s) | 1.48 (s) | 1.22 (s) |
| 27-$CH_3$ | 1.16 (s) | 1 18 (s) | 1.53 (s) | 1.51 (s) | 1.44 (s) | 1.51 (s) | 1.22 (s) |
| 29-$CH_3$ | | | 1 26 (d 6.5) | 1.26 (d 6.5) | | 1.26 (d 6) | 1.25 (d 6.6) |
| 3-CH | | 4 07 (bs) | 4.08 (bs) | 4.07 (bs) | 4.08 (bs) | | |
| 4e-CH | | 2.24 (dd 3.75,10) | | | | | |
| 5-CH | | 2.55 (dd 3.75,12.5) | 2.56 (dd 4.2,12.3) | 2.48 (dd 4,12) | 2 56 (dd 4,12) | | 2.23 (m) |
| 7-CH | | 5.64 (bs) | | | | | |
| 16-CH | 5.46 (dt 4.2,6 7) | | 5.39 (dt 4,6.5) | 5.39 (dt 4,6.5) | 5.39 (dt 4,6.2) | 5.39(m) | |
| 28-$CH_a$ | | | 5.29 (dq 2.9,6.5) | 5.29 (dq 3,6.5) | 2.62 (dd 11.3,17) | 5.28 (dq 3,6.5) | 5.32 (dq 2.2,6.6) |
| 28-$CH_b$ | | | | | 2.38 (dd 7.8,17) | | |
| 1'-CH | 4.52 (d 7.5) | 4.47 (d 7.5) | 4.42 (d 7.3) | 4.30 (d 7.5) | 4.44 (d 7.5) | 4.51 (d 7.5) | 4.53 (d 8) |
| 2'-CH | | | | 3.18 (dd 7.5,9.5) | | | |
| 6'-CHa | 3.87 (dd 2.5,12.5) | | | 3.83 (dd 2.5,12) | | | 3.87 (dd 2.5,12.5) |
| 6'-CHb | 3.71 (dd 5,12.5) | | | 3.61 (dd 5,12) | | | 3.71 (dd 5,12.5) |
| 1"-CH | 4 56 (d 7.5) | 4.68 (d 7 5) | 4.65 (d 7.3) | | 4.65 (d 7.5) | 4.59 (d 7.3) | 4.56 (d 8) |
| 6"-CHa | 3 84 (dd 2.5,12 5) | | | | | | 3.84 (dd 2.5,12.5) |
| 6"-CHb | 3.69 (dd 5,12 5) | | | | | | 3.69 (dd 5,12.5) |
| $CH_3COO$ | 2 00 (s) | | 1 96(s) | 1.96(s) | 2.07 (s) | 1.96(s) | 2.0 (s) |
| | | | 1 99(s) | 1.99(s) | | 1.99(s) | |
| | | | 2.07(s) | 2.07(s) | | 2 06(s) | |

CH (δ 4.08, bs), the 19-$CH_3$ (δ 0.87, 3H, s) and 5-CH (δ 2.56, dd, J =12.3 and 4.2 Hz), and confirmed by CD (290 nm; Δε = -1.4, $CH_3OH$). As in the case of 3 and 4, we propose a 20(S) configuration [20], whereas those at C-24 and C-28 were not determined.

Compound 6 [$(M - H)^-$ at m/z 781 in negative ion FABMS; $^1H$ NMR : see table 1] exhibited spectral properties in agreement with the hypothesis that it was the glucoside corresponding to 5. Indeed, as can be seen from table 1, the signals of the steroid moiety of 6 are identical to those of 5. Moreover, acetylation of 6 afforded an heptaacetyl derivative (6a), whose EIMS and $^1H$ NMR spectrum (table 2) fully confirmed the above hypothesis and proved that the sugar was β-glucopyranose.

*C. geminata*. Tlc analyses of the secretion of this species (30 mg from 700 individuals) showed the presence of 2 major spots. Repetitive flash silica gel column chromatographies (eluent : increasing amounts of CHCl3-AcOEt-CH3OH 1:1:1 in diethyl ether) afforded three compounds : pure 7 (7 mg) and a mixture of 5 and of its 5α-isomer 8 (6 mg).

Compound 7 [amorphous; $C_{43}H_{68}O_{17}$; $[\alpha]_{579}$ : -5.7°, c=0.35, CH3OH] exhibited spectral properties [$(M-H)^-$ at m/z 855 in negative ion FABMS, $(M + H)^+$ at m/z 857, fragment ions at m/z 533 $(M + H - C_{12}H_{21}O_{10})^+$ and at m/z 515 $(M + H - C_{12}H_{21}O_{11})^+$ in positive ion FABMS; IR : νOH 3350 $cm^{-1}$, νC=O 1760, 1730 and 1695 $cm^{-1}$; $^1H$ NMR : table 1; $^{13}C$ NMR : experimental] indicating that it possessed the familiar β-sophorose moiety linked to a steroid aglycone (MW 532, $C_{31}H_{48}O_7$). The latter must be based on a $C_{29}$ steroid skeleton, since it contained an acetate function (δ 2.07, 3H, s). Furthermore, a comparison of the $^1H$ NMR and EIMS of 7 with those of 5 strongly suggested that the structure of the tetracyclic portion of the molecule is the same for both compounds. Moreover, both also contained a C-20(S) hydroxyl group as shown by the chemical shift of the $^{21}CH_3$ group (δ 1.33). The last two oxygen atoms belonged to a γ-lactone (νC=O 1760 $cm^{-1}$; δ 178.7) necessarily located in the side chain and accounting for the two remaining degrees of unsaturation of 7. The presence of a γ-lactone function between positions C-29 and C-25 could be established by comparison of the $^1H$ and $^{13}C$ NMR spectra of 7 with those of homoterpenylmethylketone 11, (see experimental). Examination of the $^1H$ NMR spectrum of the minor component isolated from *C. geminata* showed it to be a mixture of two compounds (ratio about 1:1), despite its giving only one spot in tlc in several different solvent mixtures. The negative ion FABMS showed only one $(M-H)^-$ ion at m/z 943, thus establishing that the two compounds are isomers. Acetylation with $Ac_2O$-pyridine led to an unseparable mixture of decaacetates. Comparison of the $^1H$ NMR spectra of this mixture with that of compound 5a (vide supra) (table 3) clearly demonstrated that one of the components must be identical to 5a, whereas the other is simply the 5α-isomer 8a. Particularly informative were the chemical shifts of the 19-$CH_3$: δ 0.87 for the 5β-isomer (5) and δ 0.77 for the 5α-isomer (8) (compare with 3).

*Chrysolina hyperici*. We also had the opportunity to reinvestigate the secretion of *C. hyperici*[8]. Besides the already reported compounds 1 and 2, we isolated a minor, more polar compound which could be identified as 9 on the basis of its spectral properties and those of its acetylated derivative 9a.

Till now, nine novel polyoxygenated steroid glycosides have been isolated from four species of *Chrysolina* beetles feeding on *Hypericum*. All these compounds show a common oxydation pattern (namely C-6, C-16, C-20 and C-25) which is rarely found in naturally occurring steroids.

Table 2 $^1$H N.M.R data of peracetylated glycosides ($CDCl_3$, TMS, 250 MHz)

| | 3a | 3b* | 5a | 6a | 8a | 9a |
|---|---|---|---|---|---|---|
| 18-$CH_3$ | 0 85 (s) | 0 88 (s) | 1 12 (s) | 1 12 (s) | 1 12 (s) | 0.94 (s) |
| 19-$CH_3$ | 0 60 (s) | 0 64 (s) | 0 88 (s) | 0 88 (s) | 0 77 (s) | 0 80 (s) |
| 21-$CH_3$ | 1 16 (s)$^{++}$ | 1 23 (s)$^{++}$ | 1 30 (s) | 1 30 (s) | 1 30 (s) | 1 28 (s) |
| 26-$CH_3$ | 1 23 (s)$^{++}$ | 1 42 (s)$^{++}$ | 1 48 (s) | 1 48 (s) | 1 48 (s) | 1 22 (s) |
| 27-$CH_3$ | 1 37 (s)$^{++}$ | 1 67 (s)$^{++}$ | + | + | 1 50 (s) | 1 22 (s) |
| 29-$CH_3$ | | | ○ | ○ | 1 24 (d 7) | 1 25 (d 6 5) |
| 3-CH | | | 3 95 (bs) | 4 0 (bs) | | |
| 5-CH | | | | | | 2 28 (m) |
| 16-CH | 5 53 (dt 4 6,7 5) | | 5 37 (dt 4 2,6 1) | 5 37 (m) | 5 38 (m) | |
| 24-CH | 3 55 (dd 6 4,7 4) | 3 67 (dd 6 4,7 4) | | | | |
| 28-CH | | | 5 25 (dq 3 5,6 2) | 5 27 (m) | 5 26 (dq 3 3,6 5) | 5 26 (m) |
| 1'-CH | 4 39 (d 7 5) | 4 39 (d 7 5) | 4 44 (d 7 7) | 4 50 (d 8) | 4 56 (d 7 5) | 4 57 (d 7 5) |
| 2'-CH | 3 75 (dd 7 5,9 7) | 3 75 (dd 7 5,9 7) | 3 72 (dd 7 7,9 3) | 4 98 (dd 8,9 5) | 3 67 (m) | 3 67 (dd 7 5,9) |
| 3'-CH | 5 37 (dd 9 7,9 7) | 5,37 (dd 9 7,9 7) | 4 94 (dd 9 5,9 5) | 5 20 (dd 9 5,9 5) | 4 94 (m) | 4 96 (m) |
| 4'-CH | 5 42 (dd 9 7,9 7) | 5 42 (dd 9 7,9 7) | 5 09 (dd 9 5,9 5) | 5 10 (dd 9 5,9 5) | 5 14 (m) | 5 13 (m) |
| 5'-CH | 3 49 (m) | 3 49 (m) | 3 64 (m) | 3 65 (m) | 3 67 (m) | 3 60 (m) |
| 6'-CHa | 4 37 (dd 2 5,12 5) | 4 37 (dd 2 5,12 5) | 4 10 (dd 2 5,12 5) | 4 13 (dd 2 5,12) | 4 10 (m) | 4 14 (dd 2.5,12 5) |
| 6'-CHb | 4 60 (dd 3 8,12 5) | 4 60 (dd 3 8,12 5) | 4 32 (dd 5,12 5) | 4 24 (dd 4,12) | 4 30 (m) | 4 35 (dd 5,12 5) |
| 1"-CH | 4 80 (d 7 7) | 4 80 (d 7 7) | 4 76 (d 7 7) | | 4 82 (d 7 5) | 4 81 (d 7 8) |
| 2"-CH | 5 2-5 4 (m) | 5 2-5 4 (m) | 5 01 (dd 7 7,9 5) | | 4 94 (m) | 4 96 (m) |
| 3"-CH | 5 2-5 4 (m) | 5 2-5 4 (m) | 5 16 (dd 9.5,9.5) | | 5 14 (m) | 5 13 (m) |
| 4"-CH | 5 24 (dd 9 7,9 7) | 5 24 (dd 9 7,9 7) | 5 14 (dd 9 5,9 5) | | 5 14 (m) | 5 13 (m) |
| 5"-CH | 3 3 (ddd 2 6,3 8,9 7) | 3 3 (ddd 2 6,3 8,9 7) | 3 64 (m) | | 3 67 (m) | 3.60 (m) |
| 6"-CHa | 4 11 (dd 2 6,12 5) | 4 11 (dd 2 6,12 5) | 4 08 (dd 2 5,12 5) | | 4 10 (m) | 4 08 (dd 2 5,12 5) |
| 6"-CHb | 4 31 (dd 3 8,12 5) | 4 31 (dd 3 8,12 5) | 4 25 (dd 5,12 5) | | 4 30 (m) | 4 25 (dd 5,12 5) |
| $CH_3$ COO | 1 67(2),1 74, 1 75,1 82 1 90,1 91,1 95 | 1 67,1 69(2), 1 76(3) 1 90,1 92,1 95 | 1 97,1 99(2), 2 01(2),2 02, 2 05(2),2 08,2 09 | 2 0(2),2 02(2), 2 07,2 08,2 14 | | 2 0,2 02(2),2 04 2 06,2 07(2),2 09 |
| RO-CO-NH-CO-$CCl_3$ | | 9 79(bs) | | | | |

* in $C_6D_6$, + obscured by water signal; ○ overlapped by impurity, ++ tentative assignments

Some of these features are reminiscent of those of ecdysones, but the insect hormone activity of our derivatives has not yet been tested. Furthermore, the reason why these *Chrysolina* beetles depart from the usual defence strategy of the genus (cardenolide production) is still a matter of speculation. It seems however that, as with the cardenolides, these compounds are produced by the beetles themselves from dietary plant sterols. Indeed, the sole steroids that could be detected in *Hypericum perforatum* are the common phytosterols stigmasterol and sitosterol[22]. A conclusive proof would require the incorporation of labelled phytosterols in these beetles. Work is in progress along these lines in our laboratory.

## Experimental

- Medium pressure chromatography (flash) was performed on Macherey Nagel Kieselgel 60 (0.04-0.063 mm). In some cases, a Merck Lichroprep Si 60 Lobar$^R$ column type A was used. Tlc. analyses were performed on Macherey Nagel silica gel plates (eluent: $CH_2Cl_2$/MeOH, 90:10, 85:15 or 80:20). The steroidal glycosides were visualized by spraying with ceric sulfate.
- The $^{13}C$ and $^1H$ NMR spectra were run on a Bruker WM250 at 62.8 and 250 MHz respectively with TMS as internal standard, and are reported in tables 1 and 2, and below.

The $^{13}C$ NMR signals in 3 and 7 were assigned by using the DEPT pulse sequence and by comparison with literature data.

- Electron impact mass spectra (EIMS) and fast atom bombardment mass spectra (FABMS) were obtained respectively on Micromass VG 7070 and VG 70S mass spectrometers.

- Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 579 nm.

- Circular Dichroism measurements were performed on a JASCO J500A spectropolarimeter.

- Gas liquid chromatography (g.l.c) of TMS-ethers of sugars was performed on a Hewlett-Packard 402 gas chromatograph equipped with a glass colum (120 x 0.5 cm) packed with 4 % OV-1 on Chromosorb W at 130°.

Acetylation reactions: the steroidal glycosides were acetylated by treatment with a 1:1 mixture of freshly distilled pyridine and acetic anhydride and left under nitrogen in darkness at room temperature for 100 hours. The reaction mixture was then diluted with water and extracted 5 times with $CH_2Cl_2$. After evaporation of the solvent *in vacuo*, the residue was flash chromatographed on a silica gel column with mixtures of hexane/ethyl acetate or ether/ethyl acetate, affording the individual acetylated compounds.

Hydrolysis reaction for sugar identification: 1.0 mg of the glycoside was heated with 0.4 ml of Kiliani mixture [9] at 100°C during one hour, under nitrogen. After cooling, water (2 ml) was added and the mixture was extracted three times with $CH_2Cl_2$. The water layer was neutralized on Amberlite IR-45, and evaporated to dryness. The residue was dissolved in pyridine (85 µl) and silylated with hexamethyldisylazane (10 µl) and trimethylsilyl chloride (5 µl) before g.l.c. analysis. The sugar was identified as glucose by co-injection with a similarly derivatized authentic sample.

Isolation and spectral data of the glycosides: For all species, the defence secretions were obtained by "milking" the beetles on bits of filter paper which were stored in methanol. The filter papers were repeatedly extracted with $CH_2Cl_2/CH_3OH$ (1:1) and the organic extracts combined and evaporated under reduced pressure. This material was then submitted to the fractionation procedures as described below. All compounds were homogeneous by t.l.c. Their $^1H$ NMR spectra are reported in table 1, and the $^1H$ NMR of their acetylated derivatives in table 2.

*C. varians*: The secretions of about 2,500 beetles (collected around Brussels and bred in the laboratory) afforded 38 mg of material which was submitted to repetitive flash column chromatographies (eluent: $CH_2Cl_2/CH_3OH$: 90: 10 to 80:20). 18 mg of 3 and 1.5 mg of 4 were obtained.

3: amorphous solid; $[\alpha]_{579}$: -36° (c=0.25, $CH_3OH$). FABMS, positive mode: 815 (M + H)+, 653 (M + H - 162)+, 491 (M + H - 2 x 162)+, 449 (M + H - 2 x 162 - 42)+, 431 (M + H - 2 x 162 - 60)+, 413 (M + H - 2 x 162 - 60 - 18)+, 395 (M + H - 2 x 162 - 60 - 2 x 18)+, 143 (side chain)+; negative mode: m/z 813 (M - H)-, 651 (M - H - 162)-, 347(M - H - 2 x 162 - side chain)-.
EIMS: m/z 431($C_{26}H_{39}O_5$; calc.: 431. 27987; meas.: 431.27973), 371, 353, 329, 287, 247, 231, 229, 143.
IR: See text; UV: end absorption; $^{13}C$ NMR: C-1: 37.3, t; C-2: 30.0, t; C-3: 77.9, d; C-4: 27.3°, t; C-5: 57.7, d; C-6: 213.4, s; C-7: 47.1, t; C-8: 38.4, d; C-9: 55.0, d; C-10: 42.2, s; C-11: 22.2, t; C-12: 40.7××, t; C-13: 44.4, s; C-14: 56.1, d; C-15: 35.2, t; C-16: 79.7, d; C-17: 65.0, d; C-18: 13.6, q; C-19 :16.0, q; C-20: 86.1, s; C-21: 26.2, q; C-22: 39.3××, t; C-23: 27.8°, t; C-24: 84.2+, d; C-25: 72.5, s; C-26: 26.5, q; C-27: 27.8, q; $\underline{CH_3}$-COO-16: 21.7, q; $CH_3$-$\underline{C}$OO-16: 172.5, s; C-1': 101.7, d; C-2': 83.6+, d; C-3': 76.5*, d; C-4': 71.6•, d; C-5': 78.4*, d; C-6': 62.8, t; C-1": 105.5, d; C-2": 76.3*, d; C-3": 76.5*, d; C-4": 71.5•, d; C-5": 77.8*, d; C-6": 62.8, t. °,•,*,**,××,+,++: assignments may be interchanged.

3a: amorphous solid; EIMS: m/z 1090 ($M^{+\cdot}$- 18), 1075, 1030, 1015, 473, 455, 439, 413, 395, 331, 289, 271, 229, 211, 169.

Derivatization of 3a with trichloroacetylisocyanate: 5 mg of 3a was left at room temperature for 5 minutes with an excess of trichloroacetylisocyanate in $C_6D_6$ in an N.M.R. tube affording quantitatively 3b.$^1H$ NMR: see table 2.

4: amorphous solid; FABMS negative mode: m/z 755 (M - H)-, 593 (M - H - 162)-, 431 (M - H - 2 x 162)-; IR and UV: see text.

*C. brunsvicensis*: The secretions of 600 beetles (collected around Brussels and reared in the laboratory) afforded 15 mg of material which was submitted to flash column chromatography (eluent $CH_2Cl_2/CH_3OH$ 90:10 to 85:15). This yielded two fractions: n°1 (5 mg) and n°2 (4 mg). Fraction n°1 was rechromatographed on a Lobar Si 60 column ($CH_2Cl_2/CH_3OH$ 97:3 to 90:10) affording 2.0 mg of 5. Fraction n°2 was flash rechromatographed ($CH_2Cl_2/CH_3OH$ 99:1 to 90:10) yielding 1.5 mg of 6.

5: amorphous solid; FABMS positive mode: m/z 945 (M + H)+, 886 (M + H - 59)+, 857 (M + H - 88)+, 797 (M + H - 88 - 60)+, 603 (M + H - 2 x 162 - 18)+; negative mode: m/z 943 (M - H)-, 883 (M - H - 60)-, 855 ( M - H - 88)-, 813 (M - H - 88 - 42)-, 781 (M - H - 162)-, 347 (M - H - 2 x 162 - side chain)-
IR: see text; UV: end absorption.
5a: amorphous solid; EIMS m/z: 619, 551, 542, 483, 473, 455, 465, 423, 405, 395, 331, 289, 285, 271, 247, 229, 211, 169.

6: amorphous solid; FABMS negative mode: m/z 781 (M - H)-, 739 (M - H - 42)-, 693 (M - H - 88)-, 651 (M - H - 88 - 42)-, 619 (M - H - 162)-, 347 (M - H - 162 - side chain)-
IR: νOH: 3500 to 3100 $cm^{-1}$, νC=O 1730 and 1700 $cm^{-1}$
νC-O: 1245 $cm^{-1}$
UV: end absorption.

6a: amorphous solid EIMS m/z 778, 752, 737, 619, 585, 525, 465, 481, 421, 404, 389, 349, 331, 284, 271, 229, 211, 169.

*C. geminata*: The secretions of 700 beetles (collected in the Vosges and Harz and reared in the laboratory) afforded 30 mg of material which was submitted to repetitive flash column chromatographies (eluent: diethylether with increasing amounts of the mixture $CHCl_3/CH_3OH$/AcOEt 1:1:1, 6/4 to 3/7) affording 7 mg of 7 and 6 mg of a 1:1 mixture of 5 and 8.

7: amorphous solid $[\alpha]_{579}$: -5.7° (c=0.35, $CH_3OH$)
FABMS positive mode: m/z 857 (M + H)+, 533 (M + H - 2 x 162)+, 515 (M + H - 2 x 162 - 18)+; negative mode: m/z 855 (M - H)-
IR: see text; UV: end absorption; $^{13}C$ NMR: C-1: 30.7+, t; C-2: 26.8++, t; C-3: 76.5, d; C-4: 30.2+, t; C-5: 55.9××, d; C-6: 218.4, s; C-7: 39.2, t; C-8: 36.1°, d; C-9: 45.3, d; C-10: 37.4°, s; C-11: 22.2, t; C-12: 40.8, t; C-13: 43.3, s; C-14: 56.1,××, d; C-15: 35.6, t; C-16: 77.9, d; C-17: 63.3, d; C-18: 15.6, q; C-19: 25.2, q; C-20: 76.2, s; C-21: 27.1++, q; C-22: 47.1, t; C-23: 24.2, t; C-24: 45.0, d; C-25: 88.9, s; C-26: 22.2, q; C-27: 28.0, q; C-28: 41.2, t; C-29: 178.7, s; $\underline{CH_3}$-C00-16: 21.6, q; $CH_3$-$\underline{C}$OO-16: 172.1, s; C-1': 101.7, d; C-2': 81.9, d; C-3': 78.5•, d; C-4': 72.0**, d; C-5': 78.5•, d; C-6': 63.2*, t; C-1": 105.1, d; C-2": 74.2, d; C-3": 78.0•, d; C-4": 71.7**, d; C-5": 78.1•, d; C-6": 63.0*, t. °,•,*,**,××,+,++: assigments may be interchanged.

Oxydation of α-terpineol to homoterpenylmethylketone

1.3 g of $NaIO_4$, 24 mg of $KMnO_4$ and 0.42 g of $K_2CO_3$ dissolved in 30 ml of water were added to 100 mg of α-terpineol dissolved in 25 ml of t-butanol. After stirring for 24 hours at room temperature, the solution was discolored by addition of sodium metabisulfite and its volume reduced by evaporation of t-butanol under reduced pressure. The resulting aqueous solution was exhaustively extracted with $CH_2Cl_2$. The organic layers were combined and evaporated to dryness. The solid residue was chromatographed on flash silicagel column (eluent: hexane/acetone 9:1) giving 30 mg of pure homoterpenylmethylketone. (tlc and glc). $^{13}C$ NMR: 21.9(C-6), 23.3(C-1'), 27.4(C-7), 30.0(C-4'), 34.8(C-2'), 41.8(C-3), 45.2(C-4), 86.6(C-5), 175(C-2), 207(C-3'). $^1H$ NMR (60 MHz): 1.28(6-$CH_3$), 1.43(7-$CH_3$), 2.18(4'-$CH_3$).

C. hyperici: The secretion from 600 individuals collected in Treignes was extracted as usually, affording 17 mg of extract that was submitted to repetitive flash silicagel column chromatography (eluent: $CH_2Cl_2$-$CH_3OH$, from 9:1 to 7:3). This afforded, besides the known compounds 1 (0.5 mg) and 2 (3.6 mg), about 2 mg of still impure 9. The latter was acetylated and the acetyl derivative 9a purified by silicagel chromatography.

9: amorphous solid; $^1H$ N.M.R.: see table 1.

9a: amorphous solid; EIMS: m/z 1135 ($M^{+\cdot}$ - 17), 1117, 1102, 1075, 1057, 1042, 1015, 973, 619, 534, 481, 421, 331, 287, 285, 271, 229, 211, 169.

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