Tetrahedron Letters, Vol. 34, No. 38, pp. 6059-6062, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Novel Steroid Glycosides as Aggregation Pheromone of The German Cockroach

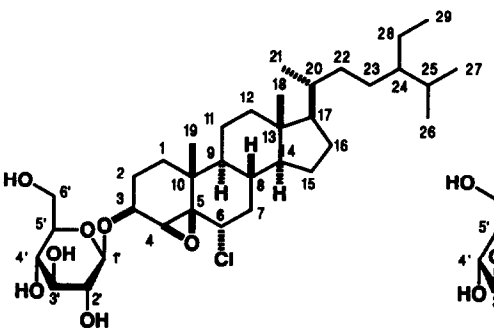
Masayuki Sakuma* and Hiroshi Fukami#

Pesticide Research Institute, Faculty of Agriculture, Kyoto University
Kitashirakawa, Sakyo-ku, Kyoto 606-01, Japan

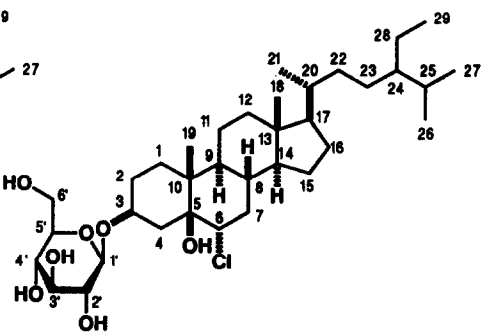
Abstract: Novel steroid glycosides, blattellastanoside A (1) and blattellastanoside B (2) were isolated from the frass-contaminated paper used as a shelter. Both 1 and 2 were unique in that they contained a chlorine atom. Both exerted arrestant activity on the German cockroach, *Blattella germanica* (L.).

The German cockroach secretes an aggregation pheromone with which it marks its harbouring site.[1] Isolation and identification of the pheromone have been the target of research and while several attempts were made,[2,3,4] no conclusive results seem to have been reached. We ascribed the difficulty to an ambiguous behavioural assay system, so we developed olfactometer [5] and choice-chamber assays [6] to discriminate between odourous attractants and arrestants of contact-chemical, respectively. From the frass-contaminated shelter paper both components were extracted with methanol, washed with n-hexane, and then were separated by solvent partitioning with n-butanol and water. Attractants in the aqueous layer were eventually identified as 1-dimethylamino-2-methyl-2-propanol and several alkylamines such as methylamine, dimethylamine, trimethylamine were found as their hydrochlorides,[7] whereas the identity of arrestants in the organic layer has remained unclear. This paper deals with isolation of the arrestant components and elucidation of the structures.

The n-butanol phase from the crude methanol extract (1,373 g) was partitioned with acidic and basic water. The neutral fraction, representing strong arrestant activity in the choice-chamber assay, was chromatographed with an ODS open column (MeOH/H_2O/trifluoroacetic acid) followed by silica gel normal phase chromatography ($CHCl_3$/MeOH). The active eluate was further purified by repeated HPLC on ODS (MeOH/H_2O, 92/8) and silica gel ($CHCl_3$/isopropanol, 90/10) to yield two dominant arrestant components **1** (15.0 mg)[8] and **2** (147.6 mg).[9]



1 (blattellastanoside A)



2 (blattellastanoside B)

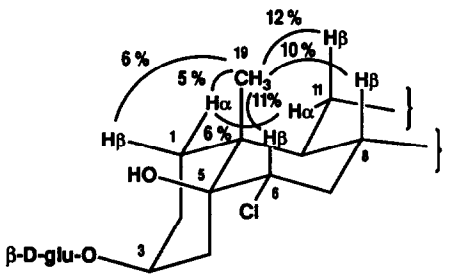
The positive ion SI-MS of the compounds showed $(M+H)^+$ ions at m/z 627 (**1**) and 629 (**2**) and the loss of 162 mu from the $(M+H)^+$ ions together with the NMR data indicated that both compounds are hexose glycosides. The glycosidic moiety was identified as β-glucopyranose by comparison of δc values of ^{13}C NMR signals in Table 1 with those of methyl β-D-glucopyranoside.[10] The elemental compositions of the aglycones were determined by high resolution EI-MS as $C_{29}H_{49}ClO_2$ (**1**) (m/z 464.3420, Δ 0.2 mmu) and $C_{29}H_{51}ClO_2$ (**2**) (m/z 466.3491, Δ 8.3 mmu), which lead to the molecular formulae *viz.* $C_{35}H_{59}ClO_7$ (**1**) and $C_{35}H_{61}ClO_7$ (**2**). Each compound contained one chlorine atom, which was supported by an accompanying isotope ion at +2 mu.

Table 1. ^1H and ^{13}C NMR Data of Compounds **1** and **2**.

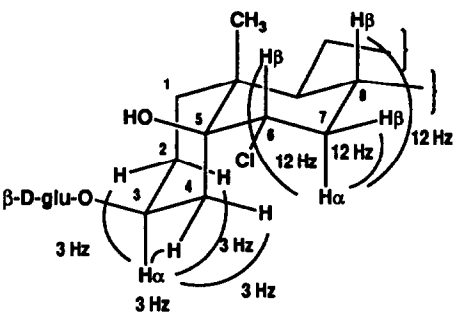
	1			**2**	
Position	δH (multiplicity, *J*)	δC(multiplicity)	Position	δH (multiplicity, *J*)	δC (multiplicity)
1α	1.57 (ddd, 13, 4, 2)	29.8 t	1α	1.88 (ddd, 13, 3, 3)	26.4 t
1β	1.24 (ddd, 13, 13, 2)		1β	1.32 (ddd, 13, 13, 3)	
2α	1.67 (dddd, 13, 13, 4, 2)	23.6 t	2α	1.54 (dddd, 13, 13, 3, 3)	25.8 t
2β	1.57 (dddd, 13, 4, 2, 2)		2β	1.79 (dddd, 13, 3, 3, 3)	
3α	4.23 (ddd, 4, 3, 2)	72.0 d	3α	4.17 (dddd, 3, 3, 3, 3)	74.7 d
4α	3.83 (d, 3)	56.0 d	4α	1.78 (dd, 13, 3)	28.8 t
4β	-		4β	2.26 (dd, 13, 3)	
5β	-	67.6 s	5β	-	76.1 s
6β	4.43 (dd, 13, 3)	58.5 d	6β	4.27 (dd, 13, 3)	67.4 d
7α	1.33 (ddd, 13, 13, 13)	41.8 t	7α	1.33 (ddd, 13, 13 13)	39.5 t
7β	2.21 (ddd, 13, 3, 3)		7β	2.05 (ddd, 13, 3, 3)	
8β	1.54 (dddd, 13, 13, 12, 3)	36.0 d	8β	1.53 (m)	35.6 d
9α	1.08 (m)	48.2 d	9α	1.23 (m)	42.9 d
10	-	38.4 s	10	-	43.0 s
11α	1.40 (dddd, 13, 3, 3, 3)	21.5 t	11α	1.41 (m)	21.6 t
11β	1.30 (dddd, 13, 13, 13, 3)		11β	1.26 (m)	
12α	1.12 (ddd, 13, 13, 3)	39.6 t	12α	1.14 (m)	39.8 t
12β	1.99 (ddd, 13, 3, 3)		12β	1.98 (ddd, 13, 3, 3)	
13	-	42.8 s	13	-	42.8 s
14α	1.08 (m)	55.6 d	14α	1.10 (m)	56.2 d
15α	1.60 (m)	24.2 t	15α	1.55 (m)	24.2 t
15β	1.09 (m)		15β	1.08 (m)	
16α	1.86 (m)	28.1 t	16α	1.84 (m)	28.2 t
16β	1.28 (m)	28.1 t	16β	1.26 (m)	28.2 t
17α	1.11 (m)	56.2 d	17α	1.10 (m)	56.2 d
18	0.67 (s, 3H)	12.0 q	18	0.65 (s, 3H)	12.0 q
19	1.03 (s, 3H)	18.7 q	19	0.94 (s, 3H)	16.9 q
20	1.34 (m)	36.2 d	20	1.32 (m)	36.2 d
21	0.91 (d, 7, 3H)	18.7 q	21	0.91 (d, 7, 3H)	18.8 q
22a	1.00 (m)	34.0 t	22a	0.99 (m)	34.0 t
22b	1.32 (m)		22b	1.32 (m)	
23a	1.13 (m)	26.4 t	23a	1.13 (m)	26.4 t
23b	1.17 (m)		23b	1.17 (m)	
24	0.93 (sextet, 8)	46.0 d	24	0.93 (sextet, 8)	45.9 d
25	1.66 (dqq, 8, 8, 7)	29.3 d	25	1.67 (dqq, 8, 8, 7)	29.2 d
26	0.84 (d, 8, 3H)	19.8 q	26	0.84 (d, 8, 3H)	19.8 q
27	0.82 (d, 7, 3H)	19.1 q	27	0.82 (d, 7, 3H)	19.1 q
28a	1.22 (m)	23.2 t	28a	1.22 (m)	23.1 t
28b	1.26 (m)		28b	1.26 (m)	
29	0.85 (t, 3H)	12.0 q	29	0.85 (t, 3H)	12.0 q
1'	4.60 (d, 7.6)	101.8 d	1'	4.46 (d, 7.6)	101.1 d
2'	3.45 (dd, 7.6, 8)	73.8 d	2'	3.44 (dd, 7.6, 8)	73.3 d
3'	3.62 (dd, 8, 8)	76.4 d	3'	3.57 (dd, 8, 8)	76.6 d
4'	3.60 (dd, 8, 8)	70.1 d	4'	3.59 (dd, 8, 8)	69.9 d
5'	3.40 (m)	75.8 d	5'	3.38 (m)	75.8 d
6'	3.86 (m)	62.2 t	6'	3.85 (m)	61.8 t

The structures of the aglycones were based on spectroscopic data. Indeed, comparison of the ^{13}C NMR spectra of **1** and **2** with those of 5α-cholestane,[11] 5β-cholestane,[11] and β-sitosteryl acetate [12] demonstrated the presence of a stigmastane skeleton in **1** and **2**. The ^{13}C NMR signals were characterized with DEPT and C-H COSY experiments (Table 1). The oxygenated or chlorinated carbons in **1** include three methine and one non-protonated carbons, whereas those in **2** are two methine and one non-protonated carbons. The constitution of carbons in **1** and **2** is completely identical except that one methylene in **2** is replaced by an oxygenated methine in **1**. The aglycone of **2** had 4 degrees of unsaturation attributable to the four ring system of the skeleton itself, and the functional groups are expected to be an alcohol, chloride, and β-D-glucopyranoside. The extra degree of unsaturation out of the 5 degrees in the aglycone of **1** can be ascribed to an ether bridge replacing the alcohol and methylene proton in **2**.

Sites of substitution in **2** were estimated on the following grounds. Comparison of the ^{13}C NMR spectral data with those of the steroid skeletons revealed that the shielding of each carbon in the D-ring and side chain is almost identical to that reported[11,12]. The C19 angular methyl signal of **2** (δc 16.9) was observed between those of 5α-cholestane (δc 12.3) and 5β-cholestane (δc 23.9). This, together with the down field shift of the C10 quarternaly carbon of **2** (δc 43.0) from those of 5α-cholestane (δc 35.5) and 5β-cholestane (δc 35.1), implies that the substitution occurred at one of the 1-CH_2, 5-CH, and 9-CH. The non-protonated carbon at δc 76.1 can be suitably assigned as C5, because the C14 shielding of **2** (δc 56.2) remained unchanged from those of 5α-cholestane (δc 56.6) and 5β-cholestane (δc 56.7) excluding the possible γ substitution effect by a hetero atom on C9. The glucose connects to C3, for the base peak ions at m/z 394 (M - $C_6H_{12}O_6$ - C_4H_6)$^+$ should be the result of *retro* Diels-Alder rearrangement in A-ring after the cleavage between the sugar and aglycone, and the connectivity between C5 and 3-CH (δH 4.17, dddd, 3,3,3,3) in COLOC spectrum specified the site. The coupling constants of this proton suggested it is in an equatorial position (Scheme 2). The similar percentage of NOE between 19-CH_3 and each of 1-CHα (5 %) and 1-CHβ (6 %) indicated *cis* connection between the A and B rings (Scheme 1), thus **2** is a 5β-isomer and the glycosidic moiety connects in 3β position. The last site is limited in either C6 or C7. The H-H COSY spectrum showed the methine proton (δH 4.27, dd, 13,3) correlating with a pair of methylene protons, and the coupling pattern of the axial proton (δH 1.33, ddd, 13,13,13) shows that it couples with a geminal and two vicinal diaxial protons (Scheme 2). The axial proton can be specified as 7-CH_α coupling with 6-CH_β, 7-CH_β, and 8-CH_β, so the hetero atom should be located in 6α position. The alcohol and chloride functions were matched with the 5β and 6α positions, and the simulation of the shieldings based on the substituent effects [13] established the structure of **2** as 1-(6α-chloro-5β-hydroxy-5β-stigmast-3β-yl)-β-D-glucopyranoside.



Scheme 1: NOESY correlation of **2**



Scheme 2: H-H COSY correlation of **2**

The spectroscopic data showed that the structure of **1** is analogous to **2** except that an oxygenated methine carbon appeared instead of the C4 methylene carbon in **2**. This means the alcohol and methylene protons in **2** are replaced by an ether bridge in **1**. The 4,5-epoxide structure could be postulated, for the methine proton (δH 3.83, d, 3) correlated with 3-CH_α (δH 4.23, ddd, 4,3,2) in the H-H COSY spectrum, and the C5 shielding of **1** (δc 67.6) shifted upfield from that of **2** (δc 76.1). The epoxide structure was confirmed by an exceptionally high J_{CH} for 4-CH (δc 56.0, J_{CH}=179.1 Hz) observed by the INEPT/N experiment. The observation of NOE between 19-CH_3 and 1-CH_2 protons revealed the *cis* connection between A and B rings in **1** as in **2**. This geometry was also reflected as a downfield shift of the C19 angular methyl shielding (δc 18.7) of **1** from that of **2** (δc 16.9), reflecting the weaker γ *gauche* effect of the epoxide function at C5 in **1** than that of the alcohol in **2**. Thus we propose the structure of **1** as 1-(6α-chloro-4β,5β-epoxy-5β-stigmast-3β-yl)-β-D-glucopyranoside.

Interpretation of NMR data allowed us to assign all the ^1H and ^{13}C signals (Table 1). Both **1** and **2** were novel compounds and were denoted as blattellastanoside A and blattellastanoside B respectively. They represented the arrestant activity as ED_{50} at 0.044 (**1**) and 3.2 (**2**) nmol / cm^2 of Whatman #1 filter paper[6]. The structures proposed for both compounds have been confirmed by synthesis.[14]

Acknowledgement: The authors wish to express their gratitude to Prof. K. Mori of the University of Tokyo and Prof. T. Ueno and Prof. Y. Kuwahara of Kyoto University for their invaluable advice. They also thank Ms. K. Ohmine and Mr. K. Shizukuishi for NMR and SI-MS measurements, respectively.

REFERENCES AND NOTES

\# Present address: Faculty of Science and Technology, Ritsumeikan University, 56-1 Toji-in, Kita-ku, Kyoto 603, Japan.

1. Ishii, S.; Kuwahara, Y. *Appl. Ent. Zool.* **1967**, *2*, 203-217.
2. Block, E.F.; McChesney, J.D. *J. Insect Physiol.* **1974**, *20*, 1683-1686.
3. Ritter, F.J.; Persoons, C.J. *Neth. J. Zool.* **1975**, *25*, 261-275.
4. McFarlane, J.E.; Alli, I. *J. Chem. Ecol.* **1986**, *12*, 1369-1375.
5. Sakuma, M.; Fukami, H. *Appl. Ent. Zool.* **1985**, *20*, 387-402.
6. Sakuma, M.; Fukami, H. *Appl. Ent. Zool.* **1991**, *26*, 223-235.
7. Sakuma, M.; Fukami, H. *Appl. Ent. Zool.* **1990**, *25*, 355-368.
8. **1**: $C_{35}H_{59}ClO_7$ $[[\alpha]_D^{20}$ -23.0 ° (c 1.0, ethanol), $(M+H)^+$ 627, $(M+Na)^+$ 649, (SI-MS), $(Agl)^+$ 464.3420 (464.3418 calcd. for $C_{29}H_{49}ClO_2$), (EI-MS)].
9. **2**: $C_{35}H_{61}ClO_7$ $[[\alpha]_D^{20}$ +3.67 ° (c 6.8, ethanol), $(M+H)^+$ 629, $(M+Na)^+$ 651, (SI-MS), $(Agl)^+$ 466.3491 (466.3574 calcd. for $C_{29}H_{51}ClO_2$), (EI-MS)].
10. Stothers, J.B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York. 1972; pp. 458-468.
11. Leibfritz, D.; Roberts, J.D. *J. Am. Chem. Soc.*, **1973**, *95*, 4996-5003.
12. Seo, S.; Tomita Y.; Tori K. *J. Chem. Soc. Chem. Comm.* **1978**. 319.
13. Blunt, E.F.; Stothers, J.B. *Org. Magn. Resonance* **1977**, *9*, 439-464.
14. Mori K.; Fukamatsu K.; Kido, M. *Liebigs Ann. Chem.*, in press.

(Received in Japan 29 January 1993; accepted 30 April 1993)