Tetrahedron Vol. 36, pp. 2133 to 2135
Pergamon Press Ltd., 1980. Printed in Great Britain

CHEMICAL STUDIES OF MARINE INVERTEBRATES—XLII[1]

THE RELATIVE AND ABSOLUTE CONFIGURATION OF DYSIDENIN[2]

C. CHARLES,[3] J. C. BRAEKMAN,[4] D. DALOZE and B. TURSCH
Université Libre de Bruxelles, Faculté des Sciences, Collectif de Bio-écologie, 1050 Bruxelles, Belgium

(*Received in UK 8 October 1979*)

Abstract—The relative and absolute configuration of dysidenin has been determined by chemical correlation between dysidenin, isodysidenin and their respective dechlorinated derivatives.

In a previous communication,[5] we reported the structure determination, including the absolute configuration, of isodysidenin (**1**), the major hexachlorinated metabolite isolated from the sponge *Dysidea herbacea* Keller collected at Laing Island (Papua-New Guinea). From the same sponge, collected at the Great Barrier Reef, Kazlauskas *et al.*[6] have isolated the closely related dysidenin, for which they proposed structure **2** without any stereochemical assignment.

On the basis of ^{1}H NMR data, we suggested that **2** is the C-5 epimer of **1**.[5] In this paper we wish to present a chemical correlation between **1** and **2** confirming this hypothesis and establishing the absolute configuration of dysidenin.

Hydrolysis of isodysidenin (**1**) with hydrochloric acid in glacial acetic acid,[7] yielded a mixture of **3** and **4** from which compound **3** was isolated as its *p*-bromophenacyl ester (**5**) and compound **4** as its 2,4-dinitrobenzene derivative (**6**) (Experimental). Likewise, hydrolysis of dysidenin (**2**) under the same conditions afforded the same compounds **3** and **4**, isolated likewise as **5** and **6**, identical in all respects with the corresponding compounds from **1**. This established that the C atoms C-2 and C-13 have the same *R* configuration in both **1** and **2**.

In addition to **3** and **4**, two other derivatives (**7a** and **7b**) were generated on acid hydrolysis of **1**. These compounds were isolated as **8a** and **8b** after treatment of the mixture with fluoro-2,4-dinitrobenzene, followed by methylation with CH_2N_2. Their physical and spectral properties (Experimental) show that these compounds are diastereoisomers having structure **8** (stereochemistry not determined) which contain the remaining C atoms C-5 to C-9 of isodysidenin. The formation of two diastereoisomers from **1** can only be explained by partial epimerisation of one of the asymmetric centres during the acid hydrolysis (C-5 or C-7). Epimerisation at both centres is ruled out since this would lead to the formation of a dl pair. Since **1** has the *S5*, *R7* configuration,[5] the diastereoisomeric pair isolated after hydrolysis must be either *S5*, *R7*+*R5*, *R7* or *S5*, *R7*+*S5*, *S7*. Moreover, the same pair of diastereoisomers is obtained from the hydrolysis mixture of dysidenin (**2**). This implies that the latter must necessarily be either *R5 R7* or *S5 S7*. A choice between these two possibilities could be done by eliminating the asymmetric centre at C-7. Reduction of the trichloromethyl groups of both **1** and **2** by a Zn–Cu couple[8] led to the reduced compounds **9** and **10** respectively. These compounds which have lost the asymmetric centres at C-2 and C-7 are still diastereoisomeric. Since it has already been demonstrated (*vide supra*) that **1** and **2** have the same *R* configuration at C-13, one must conclude that **9** and **10** have opposite configurations at C-5. It follows that dysidenin (**2**) has the absolute configuration: C-2 (*R*), C-5 (*R*), C-7 (*R*), C-13 (*R*), thus confirming our previous suggestion.[5]

Epimerisation at C-5 under the acid conditions used to perform the hydrolysis, was unexpected.† Indeed we have not been able to find any similar reaction in the literature. Moreover, if racemisations of amino acids are reported, it is always under more drastic conditions. For example, the racemisation of arginine takes place only on treatment with H_2SO_4 (50%) at 160–180° for 37 hr.[9]

Thus, since this side reaction could not be explained, we had to check very carefully the purity of dysidenin and isodysidenin before hydrolysis, in order to be sure that the formation of a diastereoisomeric mixture was not due to impure starting materials. Therefore, both compounds were submitted to extensive tlc and hplc analyses which demonstrated their homogeneity.

From a biogenetic point of view, it should be interesting to determine if the difference of configuration at C-5 between **1** and **2** results from an isomerisation taking place in the final products or if it results from the incorporation of either D- or L-trichloroleucine.

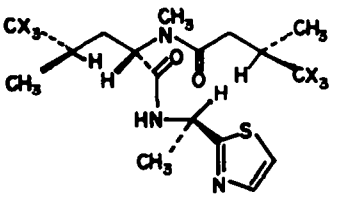
EXPERIMENTAL

The following instruments were used for measuring the physical data: IR: Pye-Unicam SP 1000; UV: Pye-Unicam SP 800; NMR: Jeol JNM/MH 100; Rotation power: Perkin–Elmer 141; MS: Finnigan 3000 D; the tlc were performed on Merck silica gel 60 F 254 plates (0.25 mm).

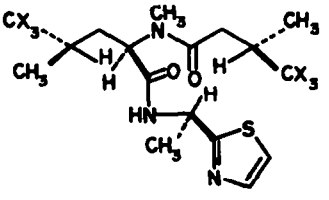
Isolation of dysidenin and isodysidenin. Sun-dried specimens of *Dysidea herbacea* (2 kg) were extracted with CH_2Cl_2. The resulting extract (120 g) was submitted to successive silica gel column chromatographies (eluent: 1. CH_2Cl_2, 2. hexane/acetone 8:2). This led to the isolation of a mixture (55 g) of isodysidenin (70%) and dysidenin (30%). Both metabolites were separated by preparative tlc, the plate being continuously eluted with $CHCl_3$ during one week. The spectral data of **2** and **1** have been previously described.[5,6]

Acid hydrolysis of isodysidenin and dysidenin. 227 mg of isodysidenin were refluxed with HCl (5 ml) in glacial AcOH (10 ml) for 20 hr. After addition of water (20 ml) the resulting soln was extracted with CH_2Cl_2. The organic layer afforded a crude acid fraction containing 66 mg of impure **3**. The aqueous layer was lyophilized and the solid residue dissolved in a 10% aqueous

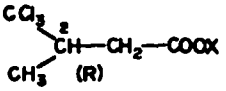
†We have checked that the epimerisation at C-5 does not occur during or after conversion of **7a** and **7b** into **8a** and **8b** respectively.

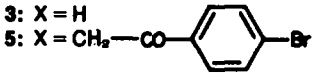


1: X = Cl
9: X = H

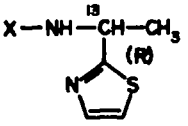


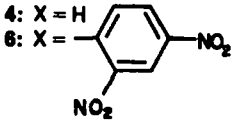
2: X = Cl
10: X = H



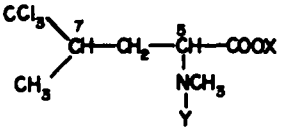


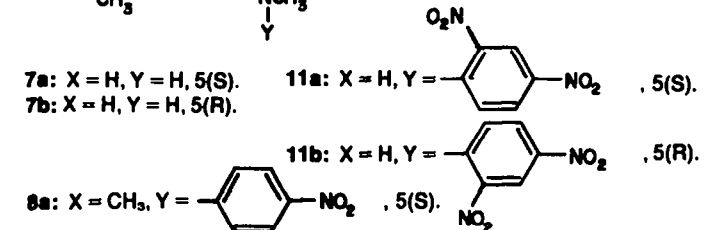
3: X = H
5: X = CH₂—CO—C₆H₄—Br





4: X = H
6: X = 2,4-(NO₂)₂C₆H₃



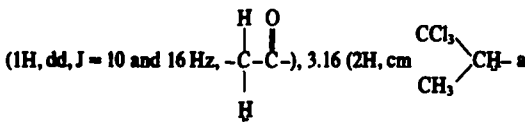


7a: X = H, Y = H, 5(S).
7b: X = H, Y = H, 5(R).
11a: X = H, Y = 2,4-(NO₂)₂C₆H₃, 5(S).
11b: X = H, Y = 2,4-(NO₂)₂C₆H₃, 5(R).
8a: X = CH₃, Y = 2,4-(NO₂)₂C₆H₃, 5(S).
8b: X = CH₃, Y = 2,4-(NO₂)₂C₆H₃, 5(R).

soln of $NaHCO_3$ (25 ml). 400 mg of fluoro-2,4-dinitrobenzene (FDNB) in EtOH (15 ml) were added to this slightly basic soln. The resulting mixture was stirred at room temp for 5 hr and extracted with CH_2Cl_2. The organic layer yielded 372 mg of a mixture containing 6 and the excess of FDNB. After acidification, the remaining aqueous layer was extracted with CH_2Cl_2. The organic layer afforded 123 mg of a crude mixture of 11a and 11b.

Hydrolysis of 150 mg of dysidenin using the same conditions yielded: 46 mg of crude 3, 84 mg of the mixture of 6 and the excess of FDNB and 51 mg of 11a + 11b.

Purification of 3. 66 mg of impure 3 originating from the hydrolysis of 1 were dissolved in warm water (5 ml), and the soln made slightly acid by addition of dil HCl. *p*-Bromophenacyl bromide (100 mg) in EtOH (15 ml) was added and the resulting mixture was refluxed for 2 hr. After evaporation of the solvent, the crude residue was submitted to a preparative tlc on silica gel (eluent: hexane/EtOAc 5%; 6 elutions). This afforded 50 mg of pure 5 ($[\alpha]_D^{22} = -7.8° \pm 0.3°$; $c = 2.35$; $CHCl_3$). Similarly, 46 mg of impure 3 originating from the hydrolysis of 2, yielded 40 mg of pure 5 ($[\alpha]_D^{22} = -8.7° \pm 0.8°$; $c = 1.15$; $CHCl_3$). 5: m.p. 95–96°; $R_f = 0.3$ (hexane/AcOEt 95:5, 3 elutions); UV (MeOH): 257 nm ($\log \epsilon = 4.26$); IR (KBr): 1755, 1705, 1590, 795 and 765 cm^{-1}; MS: characteristic ions at m/z 400 (M^+, 6), 365 (1.2), 330 (2.8), 283 (3.6), 241 (1.2), 197 (35), 183 (100) and 155 (83). ^1H NMR ($CDCl_3$/TMS, 100 MHz): 1.45 (3H, d, J = 6.5 Hz, $\underline{CH_3}$–CH), 2.56



(1H, dd, J = 10 and 16 Hz, –C(H)–C(=O)–), 3.16 (2H, cm and

–C–C–), 5.33 (2H, s, –COO–CH_2–CO–), 7.74 (AA'XX' system).

Characterization of 6. The mixture of 6 and the excess of FDNB was submitted to a silica gel column chromatography using hexane/acetone 9:1 as eluent. This afforded 85 mg of pure 6 from isodysidenin (227 mg) ($[\alpha]_{546}^{22} = +0.65 \pm 0.06°$; $c = 8.35$; $CHCl_3$) and 51 mg from dysidenin (150 mg) ($[\alpha]_{546}^{22} = +0.77 \pm 0.09°$; $c = 5.97$; $CHCl_3$).

Compound 6: m.p. 131–132°; $R_f = 0.2$ (hexane/acetone 8:2, 2 elutions); UV (MeOH): 232 nm ($\log \epsilon = 4.19$), 340 nm ($\log \epsilon = 4.29$); IR (KBr): 3340, 1628, 1594, 1586, 1522, 1505, 1420, 1338, 1300 cm^{-1}; MS: characteristic ions at m/z 294 (M^+ 25), 279 (41), 248 (85), 112 (72), 84 (100). ^1H NMR ($CDCl_3$/TMS, 100 MHz): 1.84 (3H, d, J = 6.5 Hz, $\underline{CH_3}$–CH), 5.25 (1H, quintet, J = 6.5 Hz, –NH–$\underline{CH}$–CH_3), 7.0 (1H, d, J = 9 Hz, H–C–6'), 7.36 (1H, d, J = 3 Hz) and 7.80 (1H, d, J = 3 Hz) (CH=CH of thiazole), 8.27 (1H, dd, J = 9 and 3 Hz, H–C–5'), 9.04 (1H, bd, J = 6.5 Hz, –NH–CH), 9.16 (1H, d, J = 3 Hz, H–C–3').

Esterification of the mixture of 11a + 11b. 123 mg of the crude mixture of 11a and 11b obtained from isodysidenin were dissolved in EtOH (10 ml) and treated with an ethereal soln of CH_2N_2. After elimination of the solvent, the solid residue was chromatographed on a silica gel column (eluent: hexane/EtOAc 9:1), affording 55 mg of 8a ($[\alpha]_D^{22} = -163 \pm 10°$; $c = 0.22$; $CHCl_3$) and 22 mg of 8b ($[\alpha]_D^{22} = +240 \pm 10°$; $c = 0.32$; $CHCl_3$). From dysidenin the same procedure afforded 31 mg of 8a ($[\alpha]_D^{22} = -163 \pm 10°$; $c = 0.24$; $CHCl_3$) and 26 mg of 8b ($[\alpha]_D^{22} = +258 \pm 10°$; $c = 0.35$; $CHCl_3$).

Compound **8a**: amorphous solid; $R_f = 0.35$ (hexane/AcOEt 8:2, 2 elutions); UV (MeOH): 355 nm (log $\epsilon = 4.10$); IR (film): 1755, 1610, 1525, 1340, 790 and 770 cm^{-1}; MS: characteristic ions at m/z 427 (M^+, 0.5), 392 (2.7), 368 (100), 322 (2.3) and 268 (14); ^1H NMR ($CDCl_3$/TMS, 100 MHz): 1.43 (3H, d, J = 6.5 Hz, $\underline{CH_3}$–CH), 1.90 (1H, dd, J = 15 and 7.5 Hz) and 2.78 (2H, m) ($^7\underline{CH}$–$^8\underline{CH_2}$–), 2.92 (3H, s, $\underline{CH_3}$–N<), 3.83 (3H, s, $COO\underline{CH_3}$), 4.5 (1H, dd, J = 7.5 and 7.5 Hz, –N–$\underline{CH}$–$COOCH_3$), 7.20 (1H, d, J = 9 Hz, H–C–6'), 8.33 (1H, dd, J = 9 and 3 Hz, H–C–5'), 8.72 (1H, d, J = 3 Hz, H–C–3').

Compound **8b**: amorphous solid; $R_f = 0.3$ (hexane/AcOEt 8:2, 2 elutions); UV (MeOH); 355 nm (log $\epsilon = 4.09$); IR (film): 1755, 1610, 1525, 1340, 790 and 770 cm^{-1}; MS: same as **8a**; ^1H NMR ($CDCl_3$/TMS, 100 MHz): 1.25 (3H, d, J = 6.5 Hz, –$\underline{CH_3}$–CH), 2.06 (1H, m) and 2.77 (2H, m) (^{7}CH–$^8\underline{CH_2}$–), 2.92 (3H, s, $\underline{CH_3}$–N<), 3.84 (3H, s, $COO\underline{CH_3}$), 4.42 (1H, dd, J = 13.5 Hz and 4.5 Hz, <N–$\underline{CH}$–$COOCH_3$), 7.17 (1H, d, J = 9 Hz, H–C–6'), 8.33 (1H, dd, J = 9 and 3 Hz, H–C–5'), 8.75 (1H, d, J = 3 Hz, H–C–3').

Dechlorination of **1** *and* **2** *with* Zn/Cu *couple.* 404 mg of isodysidenin were dissolved in THF (10 ml)/H_2O (2 ml) and refluxed with Zn/Cu couple (2 g) for 7 hr [The Zn/Cu couple was prepared as follows: 6.5 g of Zn dust was suspended in water (10 ml) under N_2. Acidic cupric chloride soln (0.15 M in HCl 5%, 22 ml) was added with vigorous magnetic stirring. When the evolution of gas ceased, the suspension was filtered and washed successively with water, acetone and ether. After drying under vacuum at room temp, the Zn/Cu was ready for use.]

The mixture was then filtered and extracted with CH_2Cl_2. After chromatography of the organic layer residue on a silica gel column (eluent: hexane/EtOAc 7:3), 80 mg of pure **9** were isolated. After the same procedure, dysidenin (173 mg) yielded 36 mg of **10**.

Compound **9**: amorphous solid; $R_f = 0.13$ (hexane/AcOEt 7:3, 4 elutions); $[\alpha]_D^{22°} = 133 \pm 3°$ ($c = 1.2$; $CHCl_3$); UV (MeOH): 355 nm (log $\epsilon = 4.10$); IR ($CHCl_3$): 3.400, 1685, 1635, 1520 cm^{-1}; MS: characteristic ions at m/z 339 (M^+, 6.4), 324 (2), 283 (38), 184 (100), 112 (93), 100 (96); ^1H NMR (100 MHz; $CDCl_3$/TMS): 0.94 (12H, complex signal, 2 × $\underline{CH_3}$–C$\underline{H}$–$\underline{CH_3}$), 1.62 (3H, d, J = 7 Hz), 2.24 (3H, bs), 2.93 (3H, s, $\underline{CH_3}$–N(–C=O)–), 5.36 (2H, heptuplet, J = 7.5 Hz, 2 × CH_3–$\underline{CH}$–CH_3), 7.00 (1H, bd, J = 7.0 Hz, –N$\underline{H}$–CO), 7.27 (1H, d, J = 3 Hz) and 7.73 (1H, d, J = 3 Hz) ($\underline{CH}$=$\underline{CH}$ of thiazole).

Compound **10**: amorphous solid; $R_f = 0.1$ (hexane/AcOEt 7:3, 4 elutions); $[\alpha]_D^{22°} = -122 \pm 4°$ ($c = 0.89$; $CHCl_3$); UV (MeOH): 355 nm (log $\epsilon = 4.10$); IR ($CHCl_3$): 3400, 1685, 1635, 1520 cm^{-1}; MS: identical with that of **9**; ^1H NMR ($CDCl_3$/TMS; 100 MHz): 0.93 (12H, complex signal, 2 × $\underline{CH_3}$–CH–$\underline{CH_3}$), 1.52 (3H, d, J = 7 Hz), 2.18 (3H, bs), 2.91 (3H, s, $\underline{CH_3}$–N(–C=O)–), 5.25 (2H, heptuplet, J = 7.5 Hz, 2 × CH_3–$\underline{CH}$–CH_3), 6.97 (1H, bd, J = 7 Hz, N$\underline{H}$–CO–), 7.24 (1H, d, J = 3 Hz) and 7.71 (1H, d, J = 3 Hz) (–$\underline{CH}$=$\underline{CH}$– of thiazole).

REFERENCES

[1]For part XLI, see M. Albericci, M. Collart-Lempereur, J. C. Braekman, D. Daloze, B. Tursch, J. P. Declercq, G. Germain and M. Van Meerssche, *Tetrahedron Letters* 2687 (1979).

[2]King Leopold III Biological Station, Laing Island, Papua-New Guinea, Contribution No. 18.

[3]Aspirant du Fonds National de la Recherche Scientifique.

[4]Chercheur qualifié du Fonds National de la Recherche Scientifique.

[5]C. Charles, J. C. Braekman, D. Daloze, B. Tursch and R. Karlsson, *Tetrahedron Letters* 1519 (1978).

[6]R. Kazlauskas, R. O. Lidgard, R. J. Wells and W. Vetter, *Ibid.* 3183 (1977).

[7]W. E. Bachman and M. Carmack, *J. Am. Chem. Soc.* 63, 2494 (1941).

[8]L. M. Stephenson, R. V. Gemmer and S. P. Current, *J. Org. Chem.* 42, 212 (1977).

[9]J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids* Vol. 3, p. 1853. Wiley, New York (1961).