# Structure of Aspecioside from the Monarch Butterfly Larvae Foodplants *Asclepias speciosa* and *A. syriaca*

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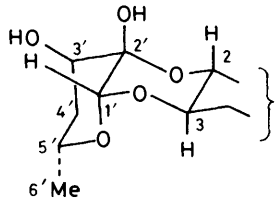
Aspecioside, a 7β,8β-epoxycardenolide glycoside isolated from *Asclepias speciosa* (Asclepiadaceae), has been shown by 400 MHz $^1$H n.m.r., $^{13}$C n.m.r., mass spectrometry, and biogenetic considerations to be 12β-hydroxy-5α-tanghinigenin-3-(6-deoxy-β-D-allopyranoside). It is also found in *A. syriaca*.

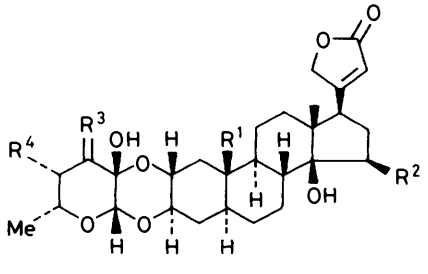
There is much evidence that the cardioactive cardenolide glycosides sequestered by monarch butterfly (*Danaus plexippus* L.) larvae from the milkweeds (*Asclepias* spp., Asclepiadaceae) provide defence for the insect against vertebrate predators in the larval, pupal, and adult stages.[1] In connection with the programme at Davis of comparing the cardiac glycoside pattern in captured monarch butterflies with those of possible larval foodplants,[2] we have determined the structure of aspecioside (**10**), a new cardenolide glycoside from *Asclepias speciosa* and *A. syriaca*, which is of some special ecological significance.[2]

Various genera of the Asclepiadaceae family, particularly *Asclepias* spp. and *Calotropis procera*, produce glycosides that are unusually stable to acid hydrolysis due to the double attachment of the carbohydrate group to the 2α and 3β positions of the cardenolide aglycone through hemiacetal and acetal links respectively.[3] Thus gomphoside (**1a**)[3] and afroside (**2a**)[4] from *A. fruticosa*, syriobioside (**4a**)[5] from *A. syriaca* and *A. speciosa*,[6] desglucosyrioside (**5a**)[5] from various *Asclepias* spp.,[2,5–7] humistratin from *A. humistrata*,[8] and various *Calotropis* cardenolides[9] all possess the doubly linked 4,6-dideoxy-β-D-hexosulose moiety (**A**), the stereochemistry of which was first determined by us in Sydney[3] and subsequently established by *X*-ray crystallography.[8,10] Variants of the carbohydrate are found in cardenolides from *Asclepias* and *Calotropis* species: *viz.* the 3′-β-D-glucoside derivative [syrioside (**5b**)[5]]; the 3′-epimer [*e.g.*, 3′-*epi*-gomphoside (**1e**)[11] and calotropin (**3e**)[9]] and its 3′-acetate (*e.g.*, asclepin[3,11,12]); the 3′-didehydro-analogue [*e.g.*, uscharidin (**3d**),[9,11] and labriformidin (**5d**)[7]] and its 3′-thiazoline [*e.g.*, uscharin (**3f**)[9,11] and labriformin (**5f**)[7]] and 3′-thiazolidine [voruscharin (**3g**)[9,11,13]] analogues. Of immediate relevance to the present work, the 4′β-hydroxy derivative of moiety (**A**) is found in calotoxin (**3c**)[9,11] and in 4′β-hydroxygomphoside (**1c**).[11] *Asclepias* cardenolides with a single linkage between the aglycone (at position 3β) and the carbohydrate are of significantly less common occurrence. Examples are ascleposide (**6**) and frugoside (**7**) which are 6-deoxy-β-D-allosides,[14,15] desglucouzarin[9] [the β-D-glucoside of uzarigenin (**8**)], and glucofrugoside.[16]

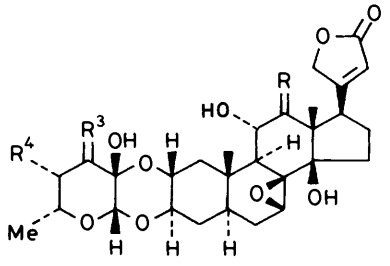
Turning to the cardenolide aglycone, structural variations found among *Asclepias* and *Calotropis* genins are, as based on the uzarigenin skeleton (**8**), oxidations at C-19 (a common occurrence in *C. procera*[9] and *A. fruticosa*[11]) and at position 15β [to produce afroside (**2a**)[4] and related glycosides[11]], dehydrogenation at 7.8 [yielding humistratin, 7,8-dehydro-(**3a**)],[8] and β-epoxidation at positions 7 and 8 accompanied by



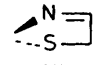
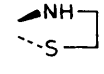
(**A**)

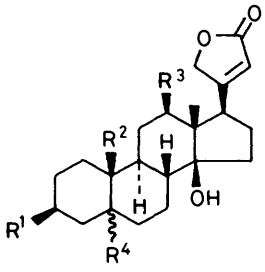


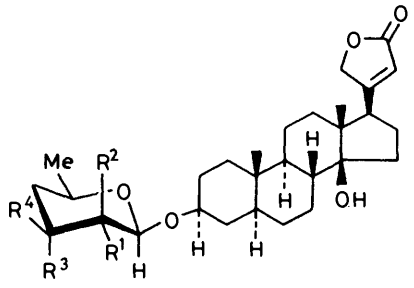
| | $R^1$ | $R^2$ |
|---|---|---|
| (**1**) | Me | H |
| (**2**) | Me | OH |
| (**3**) | CHO | H |

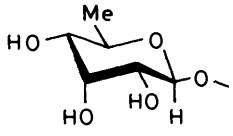


(**4**) R = α-H, β-OH
(**5**) R = O

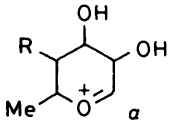
| | $R^3$ | $R^4$ |
|---|---|---|
| **a** | α-H, β-OH | H |
| **b** | α-H, β-(β-D-glucosyl) | H |
| **c** | α-H, β-OH | OH |
| **d** | O | H |
| **e** | α-OH, β-H | H |
| **f** |  | H |
| **g** |  | H |

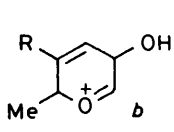


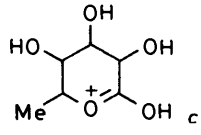


| | R¹ | R² | R³ | R⁴ |
|---|---|---|---|---|
| (6) |  | Me | H | α-H |
| (7) | | $CH_2OH$ | H | α-H |
| (8) | OH | Me | H | α-H |
| (9) | OH | Me | OH | β-H |

| | R¹ | R² | R³ | R⁴ |
|---|---|---|---|---|
| (11) | OH | H | OH | H |
| (12) | H | OH | OH | H |
| (13) | H | OH | H | OH |

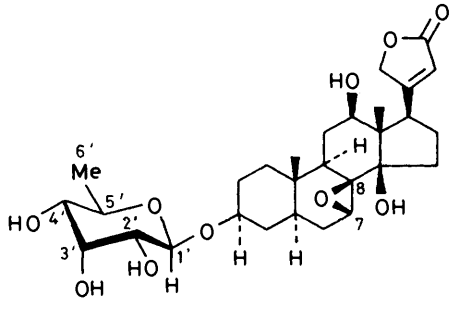

a


b


c

oxidations at positions 11 and 12 [yielding the *Asclepias* glycosides (**4a**), (**5a**), (**5b**),[5,9] (**5d**), and (**5f**)[7]].

Syriobioside (**4a**) and a new cardenolide glycoside aspecioside (**10**) were isolated from 1.8 kg of the dried, ground aerial parts of



(10) Aspecioside

*A. speciosa* from California in 0.5 × $10^{-3}$ and 1.2 × $10^{-3}$ % yield respectively. A smaller sample of *A. syriaca* from Massachusetts gave the same cardenolides in somewhat higher yields. Identical cardenolides were isolated from wings of monarch butterflies collected at Michoacan, Mexico, in yields which are an order of magnitude higher.[2]

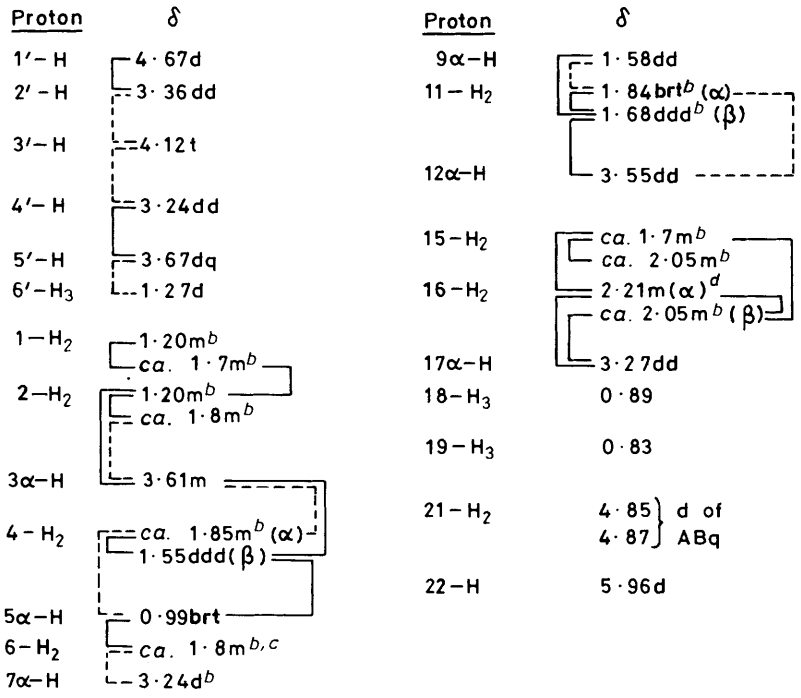
The desorption isobutane chemical ionization (c.i.) and caesium fast-atom bombardment (f.a.b.) mass spectra of aspecioside each showed a strong quasimolecular ion at *m/z* 551, a fragment ion at *m/z* 405 corresponding to the protonated genin, and characteristic ions derived from the latter by successive loss of 1—3 molecules of water. Originating from the sugar group are ions in the c.i. spectrum at *m/z* 147 and 129 (accompanied by weaker analogues 2 a.m.u. lower). These are reminiscent of the corresponding ions [*a* and *b* (R = H)] in the methane c.i. spectra of the three isomeric 3-glycosides (**11**)—(**13**) derived chemically from gomphoside (**1a**) and 3′-epigomphoside (**1e**),[17,18] and are assigned structures *a* and *b* (R = OH). An ion at *m/z* 163 in the c.i. spectrum of aspecioside is given structure *c*. The above results suggest that aspecioside consists of a 6-deoxyhexose attached by a single glycoside bond to a highly oxygenated cardenolide genin.

The structure and relative stereochemistry of the carbohydrate group in aspecioside (**10**) was readily shown by 400 MHz $^1$H n.m.r. measurements wherein all the vicinal protons in the sugar were interlinked by mutual decouplings. The magnitude of the vicinal coupling constants given in Table 2a shows that all the substituents on the pyranoside ring except the 3′-hydroxy group are equatorial. In particular, the 7.9 Hz *trans* diaxial coupling between the anomeric proton and the adjacent 2′-H establishes that aspecioside is a β-glycoside. The presence of a 14β-hydroxy-5α-card-20(22)-enolide skeleton is indicated by the appropriate $^1$H and/or $^{13}$C n.m.r. signals (Tables 1 and 3) for the system. By mutual $^1$H–$^1$H decouplings at 400 MHz, all but one of the protons in the genin were located (Table 1) and many of the coupling constants determined (Table 2b). There are 4 downfield signals originating from the genin: a multiple at δ 3.61 (half-height-width $w_{\frac{1}{2}} \geqslant 22$ Hz) assigned to an axial proton at C-3 to which the sugar is attached, a doublet of doublets at δ 3.55 (*J* 3.9, 11.5 Hz) due to an axial carbinol proton at 12α, an apparent triplet at δ 3.27 (broadened by allylic coupling) due to 17-$H_\alpha$ ($J_{16\alpha,17} + J_{16\beta,17}$ 15.2 Hz), and an apparent doublet at δ 3.24 (*J* 5.9 Hz). The origin of the signals for 12-$H_\alpha$ and 17-$H_\alpha$ is confirmed by nuclear Overhauser effect (n.O.e.) measurements which showed that irradiation of either proton led to small enhancement of the signal of the other. The shape of the last of the four signals cited above is reminiscent of that of 7-$H_\alpha$ in the 7β,8β-epoxycardenolide labriformidin (**5d**) (broad doublet at δ 3.46, *J* 5 Hz).[7]

The positions of the oxygen functions at carbons 12, 7, 8, and 3 were unambiguously confirmed by decoupling experiments at 400 MHz, which established 'connectivities' between 12-$H_\alpha$ and C-8, and between 7-$H_\alpha$ and 3-$H_\alpha$. Thus saturation of the 12-$H_\alpha$ signal caused removal of a *ca.* 13 Hz coupling to 11-$H_\beta$(ddd at δ 1.68, *J ca.* 13 Hz each), and of a *ca.* 4 Hz coupling to 11-$H_\alpha$ (broad d at δ 1.84, *J ca.* 11 Hz).* Irradiation of the protons at 11β and 11α in turn caused the collapse of respectively 2.5 and 13.0 Hz splittings in the signal of 9-$H_\alpha$ (dd at δ 1.58). As 9-$H_\alpha$ has no couplings other than to the 11-protons, C-8 is thus shown to be quaternary, in agreement with the presence of a 7,8-epoxide. Evidence for the β-configuration of this epoxide comes from $^{13}$C

* All decoupling experiments described on this paragraph were carried out reciprocally; *J′* refers to apparent coupling constant.

**Table 1.** 400 MHz $^1$H Chemical shifts of aspecioside (10)[a]




[a] Determined in 10:1 v/v $CDCl_3$–$CD_3OD$, with δ values relative to $SiMe_4$. *Mutual* decouplings are denoted by lines linking signals, full lines and dotted lines referring to collapse of 'big' (> 7 Hz) or 'small' (≤ 7 Hz) couplings respectively. Symbols d, t, q, m, and br denote doublet, apparent triplet, quartet, multiplet, and broad respectively. [b] Partly masked by other signals. [c] Signal of one of 6-H not observed. [d] N.O.e. observed when 17-$H_\alpha$ was irradiated. Signal collapsed to a ddd ( *J ca.* 3, 10, 10 Hz) when the 15-H signal near δ 1.7 was saturated.

**Table 2.** $^1$H–$^1$H Coupling constants (Hz) of aspecioside (**10**)

(a) Carbohydrate protons

| | 1′-H | 3′-H | 5′-H |
|---|---|---|---|
| 2′-H | 7.9 | 3.0 | — |
| 4′-H | — | 3.0 | 9.5 |
| 6′-H | — | — | 6.2 |

(b) Steroid protons

| | 3-$H_\alpha$ | 5-$H_\alpha$ | 7-$H_\alpha$ | 11-$H_\alpha$[a] | 11-$H_\beta$[a] | 17-$H_\alpha$ | 21-$H_2$[b] |
|---|---|---|---|---|---|---|---|
| 4-$H_\beta$[a] | *ca.* 12.5 | *ca.* 12.5 | — | — | — | — | — |
| 6-$H_\xi$ | — | *ca.* 12.5 | 5.9 | — | — | — | — |
| 6-$H_\xi$ | — | < 3 | < 1 | — | — | — | — |
| 9-$H_\alpha$ | — | — | — | 2.5 | 13.0 | — | — |
| 12-$H_\alpha$ | — | — | — | 3.9 | 11.5 | — | — |
| 16-$H_\alpha$ | — | — | — | — | — | *ca.* 9 | — |
| 16-$H_\beta$ | — | — | — | — | — | *ca.* 7 | — |
| 22-H | — | — | — | — | — | — | 1.6 |

[a] Geminal coupling *ca.* 13 Hz. [b] $J_{21,21}$ 18.3 Hz.

n.m.r. data given later. By saturation of the 7-$H_\alpha$ signal, one of the protons at C-6 was located near δ 1.8; the other 6-H was not located as it has a small coupling to 7-$H_\alpha$. Irradiation of the 6-H signal near δ 1.8 caused the collapse of a broad triplet signal assigned to 5-$H_\alpha$ (δ 0.99, *J'* 12.3, 12.3 Hz), yielding a broad doublet (*J* 13 Hz). By a series of decoupling experiments starting from either end, the axial α proton at C-3, the site of attachment of the carbohydrate group, is linked to 5-$H_\alpha$ *via* 4-$H_\beta$ (broad ddd at δ 1.55, *J' ca.* 12.5 Hz each) and 4-$H_\alpha$ (multiplet at δ *ca.* 1.85). With the assumption that aspecioside has a 14β-hydroxy group at a C/D-*cis* junction, the above $^1$H n.m.r. data lead to an unambiguous structure and stereochemistry for the steroid aglycone portion of aspecioside as shown in structure (**10**).

The proposed structure is supported by the $^{13}$C n.m.r. data shown in Table 3, which gives the chemical shifts of aspecioside and those of the relevant carbon of model cardenolides. Thus comparison with the shieldings of carbons 1—4 of the α-L-rhamnoside of uzarigenin (**8**)[19] confirms that aspecioside is the glycoside 5α-cardenolide, while comparison with the data of carbons 6—8 of labriformidin (**5d**)[7] (a 7β,8β-epoxy-5α-cardenolide) supports the proposed stereochemistry at positions 5, 7, and 8. Finally the presence of a 12β-hydroxy group is shown by comparison with the signals of carbons 12, 16, 17, and 18 of digoxigenin (**9**), a 12β-hydroxycardenolide.*

As discussed earlier, the relative stereochemistry of the 6-deoxyhexose group is established by the $^1$H–$^1$H coupling data (Table 2a). In Table 3, the carbon shieldings of the sugar in aspecioside (**10**) are compared with those of the 4,6-dideoxy-β-D-*arabino*-hexopyranoside (**11**) derived from gomphoside (**1a**).[17] As expected, the equatorial 4′-hydroxy group (found in the former sugar but not in the latter) has deshielding effects on the β carbons C-3′ and C-5′, a small periplanar heteroatom effect[20] on C-2′, and [as in the case of 4′-hydroxygomphoside (**1c**)] a shielding effect on the γ carbon C-6′ (−3 p.p.m.).

The present work establishes that the carbohydrate in aspecioside is a β-glycoside, but does not directly yield its

* Complete assignment of digoxigenin follows from comparison with digitoxigenin [5β-(**8**)].[19] The 12β-hydroxy group in the former gives rise to β effects on C-11 and C-13 (8.5 and 6 p.p.m.), α effects on C-17 and C-18 (5.5 and 6.5 p.p.m.), and a periplanar heteroatom effect[20] on C-9 (−3 p.p.m.).

**Table 3.** $^{13}C$ Chemical shifts δ (in p.p.m.)[a]

| Compound | (1c)[11] | (11)[17] | Aspecioside (10) | α-L-Rhamnoside of uzarigenin (8)[19] | (5d)[7] | (9) |
|---|---|---|---|---|---|---|
| Solvent | 1:1 $CDCl_3$–$CD_3OD$ | $CDCl_3$ | 10:1 $CDCl_3$–$CD_3OD$ | $CDCl_3$–$CD_3SOCD_3$ | $CDCl_3$ | 3:1 $CDCl_3$–$CD_3OD$ |
| δ($CDCl_3$) | 77.3 | 77.0 | 77.0 | 78.8 | 77.0 | 77.2 |
| C-1′ | | 100.6 | 98.2 | | | — |
| C-2′ | | 71.3 | 69.6 | | | — |
| C-3′ | 74.0 | 67.2 | 70.6 | | | — |
| C-4′ | 69.1 | 38.3 | 72.6 | | | — |
| C-5′ | 71.3 | 67.2 | 71.0 | | | — |
| C-6′ | 17.3 | 20.3 | 17.6 | | | — |
| C-1 | | | 38.0 | 37.0 | | 29.6* |
| C-2 | | | 28.6 | 29.0 | | 27.4** |
| C-3 | | | 75.0* | 74.9 | | 66.5 |
| C-4 | | | 33.5 | 33.8 | | 33.1 |
| C-5 | | | 39.4 | | | 36.0 |
| C-6 | | | 34.4 | | 35.9 | 26.5 |
| C-7 | | | 52.5 | | 53.9 | 21.5 |
| C-8 | | | 62.8 | | 62.1 | 41.1 |
| C-9 | | | 42.9 | | | 32.3 |
| C-10 | | | 34.2 | | | 35.2 |
| C-11 | | | 28.6* | | | 29.8* |
| C-12 | | | 77.4* | | | 74.6 |
| C-13 | | | 57.5 | | | 55.9 |
| C-14 | | | 80.7 | | | 85.6 |
| C-15 | | | 28.6 | | | 32.9 |
| C-16 | | | 27.9* | | | 27.5** |
| C-17 | | | 45.8 | | | 45.6 |
| C-18 | | | 9.4 | | | 9.0 |
| C-19 | | | 12.6 | 12.0 | | 23.5 |
| C-20 | | | 175.5* | | | 175.8[b] |
| C-21 | | | 73.8 | | | 74.1 |
| C-22 | | | 117.2 | | | 117.1 |
| C-23 | | | 174.8* | | | 176.2 |

[a] Data of relevant carbons only (see text) given for reference compounds previously examined by us. [b] Signal split in single-frequency off-resonance spectrum due to $J_{CCH}$. *,** *Similar* signals within a vertical column may be reversed.

absolute configuration. To our knowledge all cardenolide glycosides from *Asclepias* and *Calotropis* spp., including *A. syriaca* and *A. speciosa* from which aspecioside was isolated, are β-D-glycosides (see Introduction section). This information, taken in conjunction with the Klyne rule,[21] leads us to propose that the sugar in aspecioside is 6-deoxy-β-D-allopyranose. Aspecioside is thus 12β-hydroxy-5α-tanghinigen-3-(6′-deoxy-β-D-allopyranoside) (**10**).

## Experimental

$^1H$ and $^{13}C$ N.m.r. data were collected on a Bruker WM 400 and a JEOL FX90Q instrument respectively. Mass spectra were measured using a VG-70/70-HS double-focussing magnetic instrument. Fast-atom bombardment spectra were obtained by the Cs liquid SIMS technique[22] from a glycerol matrix using 6 kV $Cs^+$ ions.

*Isolation of Cardenolides.*—Dried aerial parts of *Asclepias speciosa* (1.8 kg) from Sierra County, California were macerated with 1:1 v/v ethanol–water. Liquid–liquid partition was then carried out between 5:1 v/v water–methanol on one hand and light petroleum (b.p. 40—60 °C), followed by diethyl ether and then chloroform on the other. The chloroform extracts were evaporated, and the residue obtained (containing 1.9 g of cardenolides from spectrometric analysis) was chromatographed over Biosil silica gel, with gradually increasing volumes of methanol (0—10%) in chloroform. The appropriate fractions were rechromatographed twice, and then subjected to preparative t.l.c. on 2 mm silica gel G plates with 3:97 v/v methanol–ethyl acetate as developer. Finally, preparative high-pressure liquid chromatography was carried out on a Whatman Partisil column. On elution with a methyl t-butyl ether-to-tetrahydrofuran gradient, aspecioside (**10**) (21.5 mg) and syriobioside (**4a**) (10 mg) were isolated. From the dried aerial parts of *A. syriaca* from Hampshire County, Massachusetts (100 g), aspecioside (**10**) (2 mg) and syriobioside (**4a**) (1 mg) were isolated in a similar manner. Samples of aspecioside from the two sources had identical $^1H$ n.m.r. spectral data and t.l.c. behaviour in two solvent systems.

## Acknowledgements

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