*Chemistry of Natural Compounds, Vol. 37, No. 6, 2001*

# TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. LXIII. CHEMICAL TRANSFORMATION OF CYCLOARTANES. IV. PARTIAL SYNTHESIS OF TROJANOSIDE A

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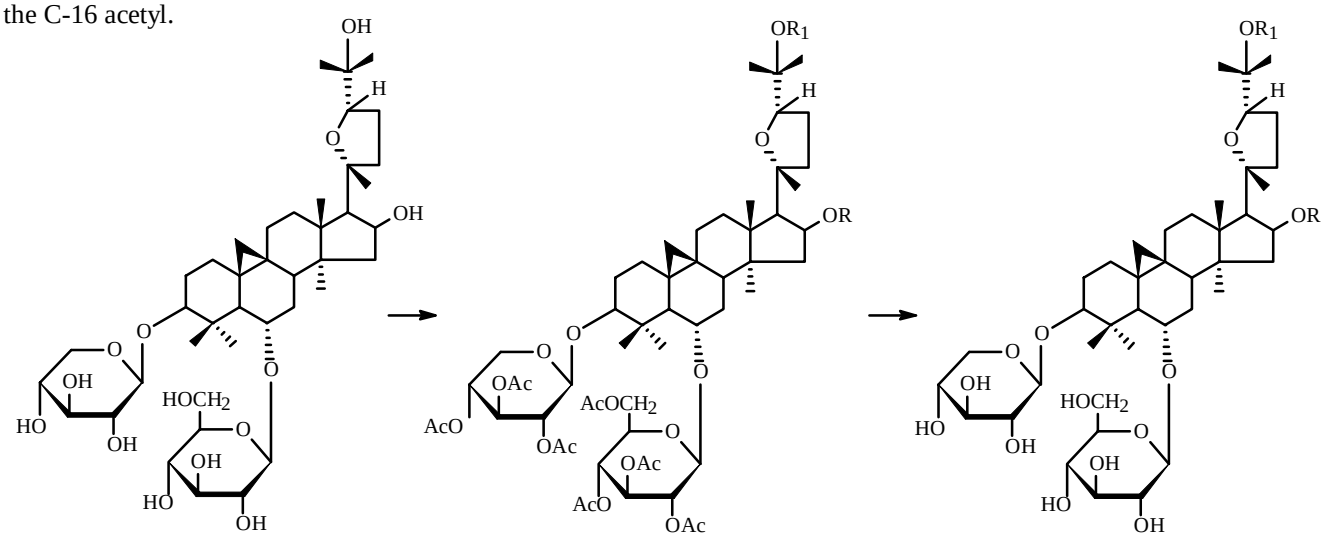
UDC 547.918:547.926

*A partial synthesis of trojanoside A, 16-O-acetyl-20R,24S-epoxycycloartan-3β,6α,16β,25-tetraol 3-O-β-D-xylopyranoside, 6-O-β-D-glucopyranoside, was developed starting from cyclosiversioside F.*

**Key words:** cycloartanes, cyclosiversioside F, trojanoside A.

The total and partial syntheses of natural compounds are reliable methods for confirming their chemical structures and offer prospects for preparing biologically active substances via synthetic or semisynthetic routes. In continuation of research on the chemistry of cycloartane methylsteroids [1], we accomplished the partial synthesis of trojanoside A (**5**), which is a natural glycoside isolated from *Astragalus trojanus* Stev. (Leguminosae) [2]. It is the 16-O-acetyl derivative of cyclosiversioside F. The synthetic scheme consists of two steps: acetylation of cyclosiversioside F (**1**) and partial deacetylation of the resulting octaacetate of cyclosiversioside F (**3**). The scheme is based on steric interference of the 16$\beta$-hydroxyl and the $\beta$-oriented C-17 side chain of cycloartane triterpenoids in addition to the involvement of this hydroxyl in the formation of an intramolecular H-bond (IHB) with other O-containing groups in the side chain. These factors are responsible for the relatively low reactivity of the hydroxyl toward acetylation by acetic anhydride in pyridine. The steric hindrance also decreases the rate of hydrolysis of the C-16 acetyl.



**1**

**2:** R = $R_1$ = Ac
**3:** R = Ac, $R_1$ = H
**4:** R = $R_1$= H

**5:** R = Ac, $R_1$ = H
**6:** R = $R_1$ = Ac

The proposed synthesis was carried out by acetylating of **1** with acetic acid in pyridine. The nonaacetate **2**, octaacetate **3**, and heptaacetate **4** were isolated from the reaction products. We identified **3** as the octaacetate of **1** that we prepared earlier [3].

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0009-3130/01/3706-0529$25.00 

TABLE 1. $^{13}C$ Chemical Shifts in **1** and **5** (δ, ppm, $C_5D_5N$, 0 = TMS)

| C Atom | Compound | | C Atom | Compound | |
|---|---|---|---|---|---|
| | **1** | **5** | | **1** | **5** |
| 1 | 32.11 | 32.04 | 24 | 81.60 | 82.82 |
| 2 | 30.09 | 30.13 | 25 | 71.23 | 70.78 |
| 3 | 88.45 | 88.43 | 26 | 26.97* | 26.74* |
| 4 | 42.54 | 42.64 | 27 | 28.48[b]* | 27.94* |
| 5 | 52.41 | 52.13 | 28 | 21.02[a] | 21.58 |
| 6 | 79.18 | 79.18 | 29 | 28.48[b] | 28.34 |
| 7 | 34.52 | 32.82 | 30 | 16.57 | 16.57 |
| 8 | 45.62 | 44.66 | | *β*-D-Xyl*p* | |
| 9 | 21.02[a] | 21.13 | 1 | 107.47 | 107.69 |
| 10 | 28.90 | 27.65 | 2 | 75.44[c] | 75.61** |
| 11 | 26.10 | 26.20 | 3 | 78.33 | 78.58[a] |
| 12 | 33.30 | 33.27 | 4 | 71.12 | 71.26 |
| 13 | 44.98 | 46.38 | 5 | 66.67 | 67.09 |
| 14 | 46.12 | 46.62 | | *β*-D-Glc*p* | |
| 15 | 46.10 | 45.08 | 1 | 105.07 | 104.95 |
| 16 | 73.36 | 76.09 | 2 | 75.44[c] | 75.57** |
| 17 | 58.12 | 57.55 | 3 | 78.98 | 78.58[a] |
| 18 | 19.76 | 19.80 | 4 | 71.73 | 71.78 |
| 19 | 28.88 | 28.71 | 5 | 77.95 | 78.21 |
| 20 | 87.17 | 85.75 | 6 | 62.95 | 62.38 |
| 21 | 28.05 | 26.61 | $\underline{CH_3}COO$ | - | 20.19 |
| 22 | 34.82 | 36.82 | $CH_3\underline{COO}$ | - | 170.25 |
| 23 | 26.36 | 26.71 | | | |

Signals marked by asterisks are not positively assigned; by the same letters, overlap.

The PMR spectrum of acetyl derivative **2** in deuteropyridine exhibits signals for nine acetyls. Therefore, this product is the peracetate of cyclosiversioside F. The PMR spectrum ($CDCl_3$) of **2** confirms this conclusion. The signal for H-16 undergoes a weak-field shift to 5.34 ppm, indicating substitution of the 16*β*-hydroxyl. The weak-field shift of the signals for two methyls in the PMR spectrum ($C_5D_5N$) of this same product **2** compared with those of acetate **3** [3] and their appearance at 1.39 and 1.49 ppm indicate that the tertiary hydroxyl in **2** is also substituted. Thus, an absorption band of hydroxyls is not observed in the IR spectrum of **2**.

The PMR spectrum of **4** contains signals for seven acetyls. This suggests that all acetyls in **4** are located on the carbohydrates whereas the C-16 and C-25 hydroxyls are intact.

It was noted previously [4] that the signal for one of the C-22 protons in PMR spectra of cycloartanes, which include the 20R,24S-epoxy-16*β*,25-diol moiety, resonates at 2.97-3.0 ppm ($C_5D_5N$, HMDS). Analysis of numerous PMR spectra shows that the signal for H-22 undergoes a significant strong-field shift if even one of these hydroxyls is substituted. The observation in the PMR spectrum of **4** ($C_5D_5N$, HMDS) of a signal for the H-22 proton at 2.96 ppm indicates that the C-16 and C-25 hydroxyls are unsubstituted. As expected, H-16 resonates at 4.84 ppm. These data together define the structure of the heptaacetate as **4**.

The octaacetate **3** was hydrolyzed by methanolic NaOH at room temperature to produce glycoside **5**. It should be noted that alkaline hydrolysis under these conditions is complete in the first minute and forms **5**. The PMR spectrum of **5** contains a 3H singlet at 2.05 ppm. This indicates that one acetyl remains in it. A triplet of doublets at 5.61 ppm with spin—spin coupling constants (SSCC) $^3J_1 = {}^3J_2 = 7.5$ Hz and $^3J_3 = 5$ Hz for H-16 unambiguously defines the location of the acetyl on C-16. The $^{13}C$ NMR spectrum of **5** is consistent with this (Table 1). Resonances for C atoms of one acetyl clearly appear at 20.19

and 170.25 ppm whereas the signal for C-16 is shifted to weak field compared with that of cyclosiversioside F and appears at 76.09 ppm.

Thus, **5** is 16-O-acetyl-20R,24S-epoxycycloartan-3$\beta$,6$\alpha$,16$\beta$,25-tetraol, 3-O-$\beta$-D-xylopyranoside, 6-O-$\beta$-D-glucopyranoside. This structure is identical to that of trojanoside A [2].

Alkaline hydrolysis of **2** formed glycoside **6**. As expected, the IR spectrum of **6** contains absorption bands characteristic of esters. Thus, the PMR spectrum of **6** exhibits signals for two acetyls at 1.94 and 1.98 ppm. The proton geminal to one of these acetyls resonates at 5.46 ppm. This signal belongs to H-16 [6] and unambiguously defines the position of one of the acetyls on C-16. Signals for two methyls in this same PMR spectrum are shifted to weak field compared with the signals of trojanoside A and appear at 1.46 ppm. Therefore, the second acetyl is located on C-25. So, glycoside **6** is cyclosiversioside F 16,25-diacetate. This also confirms the structure of cyclosiversioside F nonaacetate (**2**).

## EXPERIMENTAL

**General Coments.** Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates. Compounds on TLC were visualized using ethanolic phosphotungstic acid (25%) followed by heating for 2-5 min at 100-110°C. Column chromatography used silica gel L (Czech Rep., 50-100 μ). The solvent systems $CHCl_3$—$CH_3OH$ (100:1, 1; 50:1, 2) and $CHCl_3$—$CH_3OH$—$H_2O$ (70:23:4, 3) were used.

$^1H$ and $^{13}C$ NMR spectra were obtained on Bruker AM-400, Bruker DRX-500, and Tesla BS-567A spectrometers. $^{13}C$ NMR spectra were recorded with full C–H decoupling and with J-modulation.

IR spectra were recorded on a Perkin—Elmer System 2000 FT-IR spectrometer in KBr pellets or nujol.

**Cyclosiversioside F (1).** The source material was cyclosiversioside F isolated from roots of *Astragalus pycnanthus* Boriss. (Leguminosae), mp 284-286°C (methanol) [5].

PMR spectrum (400 MHz, $C_5D_5N$, δ, ppm, 0 = TMS, J/Hz): 0.16 and 0.52 (2H-19, d, $^2J = 4$), 0.90, 1.25, 1.25, 1.27, 1.35, 1.51, 1.90 (7×$CH_3$, s), 2.46 (H-17, d, $^3J = 7.7$), 3.02 (H-22, q, $^2J = {}^3J_1 = {}^3J_2 = 11$), 3.43 (H-3, dd, $^3J_1 = 12$, $^3J_2 = 4$), 3.61 (H-5a of D-xylose, t, $^2J = {}^3J = 11$), 3.70 (H-6, td, $^3J_1 = {}^3J_2 = 9$, $^3J_3 = 4$), 3.75-4.16 (H-24 and 7H of carbohydrates), 4.21 (H-6 of D-glucose, dd, $^2J = 12$, $^3J = 6$), 4.27 (H-5e of D-xylose, dd, $^2J = 11$, $^3J = 5$), 4.39 (H-6′ of D-glucose, dd, $^2J = 12$, $^3J = 2.5$), 4.74 (H-1 of D-xylose, d, $^3J = 7.4$), 4.79 (H-1 of D-glucose, d, $^3J = 7.7$), 4.90 (H-16, q, $^3J_1 = {}^3J_2 = {}^3J_3 = 7.7$). Table 1 lists $^{13}C$ NMR data.

**Cyclosiversioside F Nonaacetate (2), Octaacetate (3), and Heptaacetate (4) from 1.** A solution of **1** (2 g) in absolute pyridine (10 mL) was treated with acetic anhydride (10 mL), held at 15° for 12 days, and poured into icewater. The product was filtered off, washed with water, and dried. The solid was chromatographed over a column with elution by system 1 to afford **2**, 80 mg, $C_{59}H_{86}O_{23}$, mp 140-145°C (methanol). IR spectrum (ν, nujol, $cm^{-1}$): 1759-1747, 1249 (esters).

PMR spectrum (100 MHz, $C_5D_5N$, δ, ppm, 0 = HMDS, J/Hz): 0.11 and 0.43 (2H-19, d, $^2J = 4$), 0.85, 0.95, 1.14, 1.17, 1.29, 1.39, 1.49 (7×$CH_3$, s), 1.84, 1.88, 1.88, 1.90, 1.90, 2.01, 2.01, 2.07, 2.07 (9×$CH_3COO$, s), 2.47 (H-17, d, $^3J = 8$), 3.17 (H-3, m), 4.77 (H-1 of D-xylose, d, $^3J = 7$), 4.89 (H-1 of D-glucose, d, $^3J = 8$).

PMR spectrum (100 MHz, $CDCl_3$, 0 = HMDS, δ, ppm, J/Hz): 0.22 and 0.44 (2H-19, d, $^2J = 4$), 0.79, 0.89, 0.89, 0.97, 1.18, 1.36, 1.36 (7×$CH_3$, s), 1.92-2.03 (9×$CH_3COO$, s), 4.41 and 4.51 (2 anomeric protons, d, $^3J = 7$), 5.34 (H-16, m).

Continued elution of the column with the same eluent isolated amorphous octaacetate **3** (833 mg), $C_{57}H_{84}O_{22}$, which was identified by comparison with an authentic sample [3]. The PMR spectrum in $C_5D_5N$ has been published [3].

PMR spectrum (100 MHz, $CDCl_3$, 0 = HMDS, δ, ppm, J/Hz): 0.22 and 0.44 (2H-19, d, $^2J = 4$), 0.79, 0.90, 0.90, 1.02, 1.13, 1.24, 1.24 (7×$CH_3$, s), 1.92-2.01 (8×$CH_3COO$, s), 4.41 and 4.51 (2 anomeric protons, d, $^3J = 7$), 5.37 (H-16, m).

Further elution of the column by system 2 isolated heptaacetate **4** (718 mg), $C_{55}H_{82}O_{21}$, mp 230-233°C (methanol). IR spectrum (ν, KBr, $cm^{-1}$): 3474 (OH), 1759-1720, 1254-1226 (esters).

PMR spectrum (100 MHz, $C_5D_5N$, 0 = HMDS, δ, ppm, J/Hz): 0.09 and 0.41 (2H-19, d, $^2J = 4$), 0.86, 0.94, 1.17, 1.17, 1.17, 1.30, 1.44 (7×$CH_3$, s), 1.86, 1.89, 1.91, 1.91, 1.98, 2.03, 2.08 (7×$CH_3COO$, s), 2.40 (H-17, d, $^3J = 8$), 2.96 (H-22, m), 3.18 (H-3, m), 4.76 (H-1 of D-xylose, d, $^3J = 8$), 4.84 (H-16, signal overlapped by those of anomeric protons), 4.98 (H-1 of D-glucose, d, $^3J = 7$).

PMR spectrum (100 MHz, $CDCl_3$, 0 = HMDS, δ, ppm, J/Hz): 0.21 and 0.46 (2H-19, d, $^2J = 4$), 0.81, 0.89, 0.89, 1.09, 1.17, 1.17, 1.25 (7×$CH_3$, s), 1.93-2.03 (7×$CH_3COO$, s), anomeric protons: 4.42 (d, $^3J = 6$) and 4.56 (d, $^3J = 8$), 4.60 (H-16, m).

**Trojanoside A (5) from 3.** Octaacetate (**3**, 100 mg) in methanol (15 mL) was treated with methanolic NaOH (5 mL, 1%), left for 15 min at room temperature, and neutralized with methanolic HCl (7%). The solvent was evaporated. The solid was chromatographed over a column with elution by system 3 to afford **5** (70 mg), $C_{43}H_{70}O_{15}$. IR spectrum (ν, KBr, $cm^{-1}$): 3400 (OH), 3035 ($CH_2$ of cyclopropane), 1734 (bending), 1719, 1272 (ester).

PMR spectrum (500 MHz, $C_5D_5N$, 0 = TMS, δ, ppm, J/Hz): 0.15 and 0.60 (2H-19, d, $^2J = 4$), 0.93, 1.328, 1.334, 1.346, 1.349, 1.38, 1.99 (7×$CH_3$, s), 2.05 ($CH_3COO$, s), 2.58 (H-17, d, $^3J = 7.5$), 3.49 (H-3, dd, $^3J_1 = 12.5$, $^3J_2 = 5$), 3.69 (H-5a of D-xylose, t, $^2J = {}^3J = 11.3$), 3.80-3.88 (H-5 of D-glucose and H-6, m), 3.91 (H-24, t, $^3J_1 = {}^3J_2 = 8$), 3.99-4.05 (H-2 of D-glucose, H-2 and H-3 of D-xylose, m), 4.11-4.18 (H-3 and H-4 of D-glucose, m), 4.21 (H-4 of D-xylose, td, $^3J_1 = {}^3J_2 = 10$, $^3J_3 = 5$), 4.29 (H-6 of D-glucose, dd, $^2J = 12.5$, $^3J = 5$), 4.34 (H-5e of D-xylose, dd, $^2J = 10$, $^3J = 5$), 4.45 (H-6′ of D-glucose, dd, $^2J = 12.5$, $^3J = 2.5$), 4.83 (H-1 of D-xylose, d, $^3J = 7.5$), 4.87 (H-1 of D-glucose, d, $^3J = 7.5$), 5.61 (H-16, td, $^3J_1 = {}^3J_2 = 7.5$, $^3J_3 = 5$). Table 1 lists $^{13}C$ NMR data.

**Cyclosiversioside F 16,25-diacetate (6) from 2.** Peracetate **2** (25 mg) in methanol (5 mL) was treated with methanolic NaOH (1 mL, 1%), left for 15 min at room temperature, and neutralized with HCl. The solvent was evaporated. The solid was chromatographed over a column with elution by system 3 to afford **6** (14 mg), $C_{45}H_{72}O_{16}$, mp 248-252°C (methanol). IR spectrum (ν, KBr, $cm^{-1}$): 3438 (OH), 3039 ($CH_2$ of cyclopropane), 1743-1706, 1270-1249 (esters).

PMR spectrum (100 MHz, $C_5D_5N$, 0 = HMDS, δ, ppm, J/Hz): 0.04 and 0.48 (2H-19, d, $^2J = 4$), 0.84, 1.24, 1.24, 1.24, 1.46, 1.46, 1.86 (7×$CH_3$, s), 1.94, 1.98 (2×$CH_3COO$, s), 4.68 (H-1 of D-xylose, d, $^3J = 6$), 4.76 (H-1 of D-glucose, d, $^3J = 6$), 5.46 (H-16, m).

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