Phytochemistry, Vol. 23, No. 12, pp. 2931–2935, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

TWO PREGNANE ESTER GLYCOSIDES FROM *PERGULARIA PALLIDA*

NAVEEN K. KHARE, MAHESHWARI P. KHARE and ANAKSHI KHARE

Department of Chemistry, University of Lucknow, Lucknow, India

(*Revised received* 10 *April* 1984)

Key Word Index—*Pergularia pallida*; Asclepiadaceae; pallidine; pallidinine; steroid; pregnane ester mono and diglycoside.

Abstract—Two new pregnane ester glycosides designated as pallidine and pallidinine have been isolated from the dried twigs of *Pergularia pallida*. Chemical and spectroscopic evidences are consistent with the structure 12,20-di-*O*-benzoyl sarcostin-3-*O*-*β*-D-oleandroside and 12,20-di-*O*-benzoyl-sarcostin-3-*O*-*β*-D-cymaropyranosyl(1 → 4)-*β*-D-oleandropyranoside for pallidine and pallidinine respectively.

INTRODUCTION

In the course of our chemical investigation on Asclepiadaceae plants we have now focussed our attention on *Pergularia pallida* (Wight and Arn) an uninvestigated twining shrub fairly widespread in tropical India. In the present investigation of shade dried twigs of this plant, a mixture of glycosides of 2-deoxy sugars was extracted which by column chromatography over silica gel afforded two crystalline glycosides. We now report the structure of these glycosides, one of which is a pregnane ester monoglycoside (**1**) and the other its diglycoside (**7**). Only a few oligoglycosides consisting exclusively of 2-deoxy sugars are known [1] although many monoglycosides of these sugars are reported in the literature [2].

RESULTS AND DISCUSSION

Pallidine (**1**) mp 108–112°, $[\alpha]_D$ + 20°, $C_{42}H_{54}O_{11}$, was isolated by column chromatography of the diethyl ether soluble extract. It displayed positive xanthydrol [3, 4] and Keller–Kiliani reactions [5] characteristic of 2-deoxy sugars. In addition, the presence of a doublet at δ1.30 (J = 6 Hz) for a secondary methyl group besides the characteristic methylene signals in the regions δ2.4–2.2 (1H) and 2.12–1.66 (1H) for the equatorial and axial protons, respectively, in the ^{1}H NMR spectrum of **1** suggested it to be a monoglycoside of 2,6-dideoxy hexose. Besides, the ^{1}H NMR spectrum also displayed ten aromatic proton signals in the region δ7.9–7.3 for two phenyl groups which were in conformity with its UV maximum at 282 nm (log ε3.27) [6]. To identify the sugar and genin units of **1** it was hydrolysed with mild acid (0.05 N H_2SO_4) [7] which afforded an amorphous genin **3**, $[\alpha]_D$ + 81° and sugar **6**, $[\alpha]_D$ − 15°. The characteristic colour reactions for 2-deoxy sugar, exhibited by **6** and comparison of optical rotation and mobility on PC and TLC with an authentic sample led to its identification as D-oleandrose [8, 9] (2,6-dideoxy-3-*O*-methyl-D-arabinohexose). For further characterization, the sugar **6** was oxidized with bromine water to the known oleandrono-1,4-lactone obtained as syrup and identified by PC comparison with an authentic sample. This lactone afforded a crystalline phenylhydrazide, mp 133–136°, identical in properties with D-oleandronic acid phenylhydrazide [8, 9].

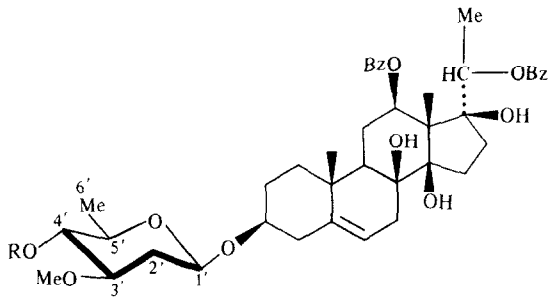
Identification of D-oleandrose as the sugar of the glycoside **1** gave the formula $C_{35}H_{42}O_8$ for its aglycone **3**. The aglycone, in turn underwent saponification with methanolic potassium hydroxide, and afforded sarcostin (**4**) [10] $C_{21}H_{34}O_6$ as the genin. The formula $C_{14}H_8O_2$ obtained by the difference of the molecular formulae of the aglycone **3** and the deacylated product sarcostin (**4**) indicated the size of the acyl group(s). These acyl groups could be two benzoyl groups as manifested by the ^{1}H NMR and UV spectra of the glycoside **1**. Two hydroxyl groups of sarcostin were thus benzoylated and the third one was in glycosidic linkage with the oleandrose.

Sarcostin had therefore no free acylable hydroxyl group in pallidine. As neither the glycoside **1** nor sarcostin dibenzoate (**3**) obtained from it, reacted with sodium periodate, it was evident that one of the ester groupings is definitely present at C-20. The aglycone **3** could thus either be 3,20-di-*O*-benzoyl sarcostin or 12,20-di-*O*-benzoyl sarcostin. A comparison of the optical rotation ($[\alpha]_D$ + 81.2°) of this amorphous di-*O*-benzoyl sarcostin (**3**) identified it as the reported amorphous 12,20-di-*O*-benzoyl sarcostin [6]. On the basis of chemical investigation and characterization of the hydrolysis product, pallidine (**1**) was concluded to be 12,20-di-*O*-benzoyl sarcostin-3-*O*-D-oleandroside.

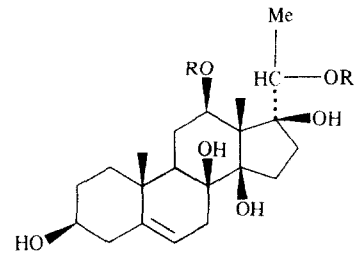
Acetylation of the glycoside **1** with acetic anhydride in pyridine yielded only an amorphous monoacetate **2**, $[\alpha]_D$ + 10°, characterized from its ^{1}H NMR spectrum. The formation of this monoacetate obviously occurred at the C-4′ hydroxyl group of the oleandrose moiety of **6**.

Its mass spectrum did not exhibit an $[M]^+$ but the highest mass ion recorded at m/z 346.2137 was in agreement with the formula $C_{21}H_{30}O_4$ which corresponded to $[M - 2C_6H_5COOH - \text{oleandrose}]^+$. The subsequent losses of three water molecules from this ion are in support of the three unacetylable tertiary hydroxyl groups of its sarcostin moiety. The low mass region contained the common 2,6-dideoxy monomethoxy hexose fragments [11] at m/z 145, 130, 113, 95 and 86.

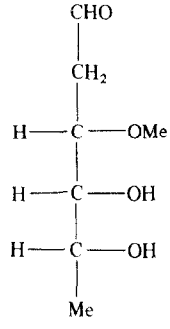
The ^{1}H NMR spectrum of the glycoside **1** at 400 MHz not only confirmed the derived structure but also defined



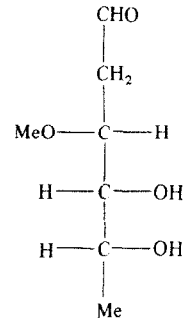
R
1 H
2 Ac



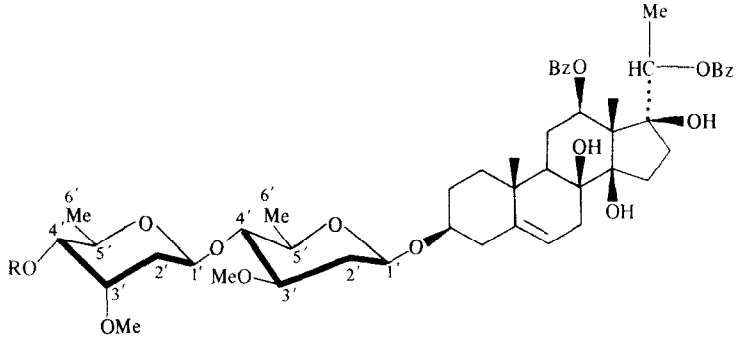
R
3 Bz
4 H



5



6



R
7 H
8 Ac

the configuration of the glycosidic linkage. The anomeric proton was observed as a double doublet at δ4.94 (J = 8 and 1.5 Hz). The large value of the coupling constant (J = 8 Hz) was typical of an axial anomeric proton of a 2-deoxy hexopyranoside, which suggested that it was present in the $^4C_1$ (D) conformation and was joined to the aglycone through a β-glycosidic linkage [12]. A sharp three proton singlet at δ3.50 was attributed to the methoxy group. Three one proton multiplets in the region δ3.81, 3.62 and 3.18 could be assigned to the methine protons H-5', H-3' and H-4', respectively, of the sugar moiety.

The ^{1}H NMR spectrum also displayed characteristic signals of the genin moiety showing C-19 and C-18 tertiary methyl group singlets at δ1.14 and 1.27, respectively, and an additional six proton doublet at 1.30 (J = 6 Hz) of the C-21 secondary methyl group submerged under the 6-deoxy secondary methyl group of the sugar unit. The one proton multiplet at 5.38 was assigned to the vinylic proton at C-6. A quartet of one

proton at 4.87 ($J = 6$ Hz) was due to the methine proton at C-20 and a double doublet at 4.67 (1H, $J = 8$ and 1.5 Hz) was typical of an axial methine proton at C-12 which was confirmed by irradiation experiments. Irradiation of this double doublet at 1866 Hz resulted in a collapse of the aglycone methylene proton signals in the region 1.66–1.38. Ten aromatic proton multiplets in the region 7.9–7.3, in conjunction with the mass spectral peak at m/z 346.2137 $[M - 2C_6H_5COOH - \text{oleandrose}]^+$ and a strong peak at m/z 105.0348 $[C_6H_5CO]^+$ again confirmed the presence of two benzoate groups in the glycoside.

On the basis of the foregoing facts pallidine (**1**) was found to be 12,20-di-*O*-benzoyl sarcostin-3-*O*-*β*-D-oleandroside.

Pallidinine (**7**), mp 118–122°, $[\alpha]_D$ +88°, $C_{49}H_{66}O_{14}$, was also isolated by column chromatography of the diethyl ether soluble glycoside over silica gel. It gave positive tests in xanthydrol [3, 4] and Keller–Kiliani [5] reactions indicating the presence of 2-deoxy sugars. The characteristic lower field two methylene group signals in the regions 2.0–1.68 (2H) and 2.47–2.1 (2H) in conjunction with a doublet ($J = 6$ Hz) for two secondary methyl groups at 1.38 (6H) in the ^{1}H NMR spectrum suggested **7** to be a diglycoside of 2,6-dideoxy hexoses.

Mild acid hydrolysis of **7** with 0.05 N H_2SO_4 afforded an amorphous genin **3**, $[\alpha]_D$ +78° and a mixture of two sugars. The separated sugars **5** and **6** displaying characteristic colour tests of 2-deoxy sugars were identified as D-cymarose [13] (2,6-dideoxy-3-*O*-methyl-D-ribohexose) and D-oleandrose (2,6-dideoxy-3-*O*-methyl-D-arabinohexose) by direct comparison (PC and $[\alpha]_D$) with the authentic samples. Further characterization of these sugars was achieved by their oxidation with bromine water to D-cymarono-1,4-lactone and D-oleandrono-1,4-lactone which on treatment with phenylhydrazine afforded crystalline D-cymaronic acid phenylhydrazide [13], mp 151–153° and D-oleandronic acid phenylhydrazide, mp 133–135° identified by comparison (IR and mmp) with authentic samples. On the basis of the above results, **7** was inferred to be a diglycoside of D-cymarose and D-oleandrose.

A more direct chemical support for **7** being a diglycoside of cymarose and oleandrose and their sequence came from its very mild acid hydrolysis (0.005 N H_2SO_4) which also helped in establishing the relationship of **7** and **1** by affording partially hydrolysed products. After three days an aliquot of partially hydrolysed mixture (PC) when preparatively worked up to afford only two products **1** and **5** besides some unreacted starting material **7**. The polar component **5** was obtained as an amorphous product $[\alpha]_D$ +50° whereas the less polar component was a crystalline product **1**, mp 108–112°, $[\alpha]_D$ +21°. Product **5** was identified as D-cymarose by comparison of its optical rotation and co-chromatography on PC with an authentic sample. Compound **1** was found to be identical with the other isolated glycoside pallidine by comparing its optical rotation, mp, mmp and TLC properties. The hydrolysis was complete in 92 hr (PC) and working up of the hydrolysate followed by chromatography on a silica gel column afforded three chromatographically pure, amorphous products **3**, **5** and **6**. The two more polar reducing amorphous compounds **5** and **6** exhibiting colour reactions of 2-deoxy sugars were identified as D-cymarose and D-oleandrose, respectively, by comparing their optical rotation and PC properties with authentic samples. The other non-reducing amorphous product **3** was 12,20-di-*O*-benzoyl sarcostin isolated earlier from pallidine. Its alkaline hydrolysis yielded crystalline sarcostin (**4**) (identified by TLC, IR and mmp).

As cymarose was the first sugar unit obtained along with the glycoside pallidine in the partial acid hydrolysis of **7**, it led to the conclusion that cymarose was the terminal sugar and the other sugar unit oleandrose is glycosidically linked to the C-3 hydroxyl group of 12,20-di-*O*-benzoyl sarcostin moiety.

The mass spectrum of **7**, like that of pallidine, did not exhibit the $[M]^+$ ion but the highest recorded ion at m/z 328.2058 corresponded to the molecular formula $C_{21}H_{28}O_3$ relating it to $[M - 2C_6H_5COOH - \text{sugars} - H_2O]^+$.

The ^{1}H NMR spectrum of **7** at 400 MHz is in full agreement with the derived structure indicating further a *β*-D-cymaropyranoside linkage for the end sugar. Ten aromatic protons in the region δ 7.94–7.3 were due to two benzoate groups. A two proton double doublet at 4.96 ($J = 8$ and 2 Hz) could be assigned to two anomeric protons of the two sugars, the configuration of C-1′ in both being identical. Its large coupling constant ($J = 8$ Hz) was typical of an axial proton on a 2-deoxy hexopyranose in the $^4C_1$ (D) conformation also suggesting that both the sugar units were linked through a *β*-D (1 → 4) glycoside linkage. The assignment of these two axial anomeric proton signals is also in agreement with the results of the irradiation experiment. Irradiation of the axial proton of the methylene group of 2-deoxy sugars at 692 Hz led to the collapse of this double doublet to a broad singlet whereas the irradiation of the equatorial proton of the methylene group at 842 Hz resulted in the collapse of this double doublet to a doublet ($J = 8$ Hz). In the higher field, a six proton doublet centered at 1.38 ($J = 6$ Hz) was attributed to the two secondary methyl groups and another sharp six proton singlet at 3.42 was assigned to the methoxy groups of the two sugar units.

In the light of the foregoing evidence, the structure of pallidinine (**7**) was established as 12,20-di-*O*-benzoyl sarcostin-3-*O*-*β*-D-cymaropyranosyl(1 → 4)-*β*-D-oleandropyranoside which was also supported by the result of its acetylation with acetic anhydride in pyridine which gave an amorphous monoacetate **8**, $[\alpha]_D$ +47°.

EXPERIMENTAL

Mps were determined on a Boetius micromelting point apparatus and are uncorr. Sugars were made visible with 50% aq. H_2SO_4 on TLC and vanillin–$HClO_4$ reagent [14] on PC. PC was performed using $C_6H_5CH_3$–BuOH (4:1) satd with H_2O. ^{1}H NMR spectra ($CDCl_3$) were recorded on a 400 MHz (Bruker), 90 MHz (Perkin–Elmer R-32) and 80 MHz (CFT-20, proton probe) spectrometers with TMS as internal standard. MS were recorded with a JEOL high resolution JMS-300 mass spectrometer. $[\alpha]_D$ were measured in a 1-dm tube with a Jasco-Dip 180 automatic polarimeter.

Plant extraction. The twigs of *P. pallida* (25 kg) were collected from Mussorie, India. The identity of the plant was confirmed by Dr. S. L. Kapoor, Systemic Botanist, National Botanical Research Institute, Lucknow (India), where a voucher specimen was deposited. Shade dried powdered twigs (4 kg) were extracted by the method used for pregnane glycosides [6], using 50–95% aq. EtOH. The ethanolic extracts were concd under red. pres. and the concentrate was exhaustively extracted successively with petrol, Et_2O, $CHCl_3$, $CHCl_3$–EtOH (4:1) and $CHCl_3$–EtOH

(3:2). These extracts on evaporation yielded the following quantities of residues: petrol, 2.5 g; Et_2O, 1 g; $CHCl_3$, 10 g; $CHCl_3$–EtOH (4:1), 7 g; $CHCl_3$–EtOH (3:2), 2.5 g. The last four extracts were rich in glycosides (xanthydrol test positive). Repeated column chromatography of the Et_2O extract using $CHCl_3$–MeOH (98:2) as eluent afforded pallidine (**1**) (62 mg) and pallidinine (**7**) (55 mg).

Pallidine (**1**). Mp 108–112° (Me_2CO–petrol), $[\alpha]_D^{25}$ +20.4° (MeOH, *c* 0.11), (Found: C, 68.98; H, 7.06. $C_{42}H_{54}O_{11}$ requires C, 68.66; H, 7.35%). It gave positive tests in the xanthydrol and Keller–Kiliani reactions. UV λ_{max}^{EtOH} nm (log ε): 282 (3.27). ^{1}H NMR (400 MHz): δ7.9–7.3 (10H, aromatic), 5.38 (1H, *m*, H-6), 4.94 (1H, *dd*, *J* = 8 and 1.5 Hz, H-1′), 4.87 (1H, *q*, *J* = 6 Hz, H-20), 4.67 (1H, *dd*, *J* = 8 and 1.5 Hz, H-12), 3.81 (1H, *m*, H-5′), 3.62 (1H, *m*, H-3′), 3.50 (3H, *s*, OMe), 3.18 (1H, *m*, H-4′), 2.4–2.2 (1H, *m*, H-2′e), 2.12–1.66 (1H, *m*, H-2′a), 1.66–1.38 (methylenes of aglycone), 1.30 (6H, *d*, *J* = 6 Hz, 6′-Me, 21-Me), 1.27 (3H, *s*, 18-Me), 1.14 (3H, *s*, 19-Me), MS *m/z* (rel. int.): $[M]^+$ (not observed), 346.2137 (0.94) $[M-2C_6H_5COOH-\text{sugar}]^+$ ($C_{21}H_{30}O_4$), 328.2024 (1.22) $[346-H_2O]^+$ ($C_{21}H_{28}O_3$), 313.1805 (0.72) $[328-CH_3]^+$ ($C_{20}H_{25}O_3$), 310.1928 (0.74) $[328-H_2O]^+$ ($C_{21}H_{26}O_2$), 295.1698 (0.62) $[310-Me]^+$ ($C_{20}H_{23}O_2$), 292.1798 (0.53) $[310-H_2O]^+$ ($C_{21}H_{24}O$), sugar fragments: 145.0870 (100) ($C_7H_{13}O_3$), 130.0652 (2.09) ($C_6H_{10}O_3$), 113.0606 (0.62) ($C_6H_9O_2$), 95.0498 (20.45) (C_6H_7O), 86.0373 (0.57) ($C_4H_6O_2$), aglycone fragments: 138.1035 (0.76) ($C_9H_{14}O$), 120.0938 (2.10) (C_9H_{12}), 105.0348 (12.94) (C_6H_5CO).

Pallidinine (**7**). Mp 118–122° (Et_2O and petrol), $[\alpha]_D^{25}$ +88.6° (MeOH, *c* 0.07), (Found: C, 67.20; H, 7.26. $C_{49}H_{66}O_{14}$ requires C, 66.97; H, 7.51%). It gave positive tests in the xanthydrol and Keller–Kiliani reactions. UV λ_{max}^{EtOH} nm (log ε): 282 (3.24). ^{1}H NMR (400 MHz): δ7.94–7.3 (10H, aromatic), 5.39 (1H, *m*, H-6), 4.96 (2H, *dd*, *J* = 8 and 2 Hz, H-1′ of cym and ole), 4.88 (1H, *q*, *J* = 6 Hz, H-20), 4.56 (1H, *dd*, *J* = 9 and 1.5 Hz, H-12), 3.82 (2H, *m*, H-5′ of cym and ole), 3.62 (2H, *m*, H-3′ of cym and ole), 3.42 (6H, *s*, 2OMe), 3.20 (2H, *m*, H-4′ of cym and ole), 2.47–2.10 (2H, *m*, H-2′e of cym and ole), 2.00–1.68 (2H, *m*, H-2′a of cym and ole), 1.68–1.40 (methylenes of aglycone), 1.38 (6H, *d*, *J* = 6 Hz, 6′-Me of cym and ole), 1.35 (3H, *d*, *J* = 6 Hz, 21-Me), 1.24 (3H, *s*, 18-Me), 1.12 (3H, *s*, 19-Me). MS: *m/z* (rel. int.) 328.2058 (0.74) $[M-2C_6H_5COOH-\text{sugars}-H_2O]^+$ ($C_{21}H_{28}O_3$), 295.0017 (0.86) $[328-H_2O-Me]^+$ ($C_{20}H_{23}O_2$), sugar fragments: 145.0872 (100) ($C_7H_{13}O_3$), 130.0655 (6.52) ($C_6H_{10}O_3$), 113.0605 (62.89) ($C_6H_9O_2$), 95.0498 (19.14) (C_6H_7O), 86.0373 (0.57) ($C_4H_6O_2$), aglycone fragments; 179.1072 (1.22) ($C_{11}H_{15}O_2$), 137.0966 (2.18) ($C_9H_{13}O$), 120.0933 (1.85) (C_9H_{12}), 105.0345 (9.57) (C_6H_5CO).

Mild hydrolysis of **1** *with acid.* To a soln of crystalline **1** (15 mg) in 80% aq. 1,4-dioxane (1 ml) was added 0.1 N H_2SO_4 (1 ml) and the soln was warmed for 30 min at 50°, then concd under red. pres. to remove dioxane. The aq. portion was repeatedly extracted with $CHCl_3$–MeOH (97:3) and the organic layer was washed in turn with H_2O, 2 N Na_2CO_3, again with H_2O, dried over Na_2SO_4 and evapd to afford genin **3** as an amorphous residue (10 mg), $[\alpha]_D^{25}$ +81.2° (MeOH, *c* 0.21). The aq. hydrolysate was neutralized with freshly precipitated $BaCO_3$, filtered and concd under red. pres. to afford the sugar **6** (3 mg), as colourless thick syrup $[\alpha]_D^{25}$ −15.0° (H_2O, *c* 0.11). It gave a pink colouration in the xanthydrol and green colouration in Keller–Kiliani reactions. Its TLC, PC and optical rotation comparison confirmed **6** as D-oleandrose.

Mild hydrolysis of **7** *with acid.* To a soln of crystalline **7** (18 mg) in 80% aq. 1,4-dioxane (1.2 ml) was added 0.1 N H_2SO_4 (1.2 ml) as in the acid hydrolysis of **1**, affording a genin **3** as an amorphous residue (9 mg), $[\alpha]_D^{25}$ +77.9° (EtOH, *c* 0.22). The aq. hydrolysate was concd under red. pres.; a mixture of two sugars were obtained which were isolated through column chromatography over silica gel affording **5** (3 mg), $[\alpha]_D^{25}$ +49.41° (H_2O, *c* 0.11) and **6** (3 mg), $[\alpha]_D^{25}$ −10° (H_2O, *c* 0.14). Both gave a positive colouration in the xanthydrol and Keller–Kiliani reactions. The optical rotation, TLC and PC comparison of **5** and **6** showed them to be identical to D-cymarose and D-oleandrose, respectively.

Oxidation of **5** *with bromine water.* A soln of **5** (2.5 mg) in H_2O (0.4 ml) was mixed with Br_2 (7 μl) and shaken in a stoppered flask in the dark for 24 hr at room temp. The excess of Br_2 was then removed under red. pres., the acidic mixture was made neutral with freshly precipitated Ag_2CO_3 and the suspension was filtered, H_2S was passed through the filtrate to remove Ag^+ ions, and the suspension was filtered. The filtrate was evapd to dryness under red. pres. yielding syrupy lactone (2 mg) showing only one spot with the NH_2OH–$FeCl_3$ reagent and having the same mobility as D-cymarono-1,4-lactone on TLC.

Oxidation of **6** *with bromine water.* A soln of **6** (3 mg) in H_2O (0.8 ml) was mixed with Br_2 (13 μl) as in the oxidation of **5**, affording syrupy lactone (2 mg) showing only one spot with NH_2OH–$FeCl_3$ reagent and having the same mobility as D-oleandrono 1,4-lactone on TLC.

D-Cymaronic acid phenylhydrazide. A soln of D-cymarono-1,4-lactone (2 mg) in absolute EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) and the mixture was heated for 30 min at 100°. The viscous mass was cooled and repeatedly triturated with absolute Et_2O (to remove excess of phenylhydrazine) yielding a D-cymaronic acid phenylhydrazide which crystallized from MeOH–Et_2O as colourless needles (1.1 mg), mp 151–153°.

D-Oleandronic acid phenylhydrazide. A soln of D-oleandrono-1,4-lactone (2 mg) in absolute EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) as in D-cymarono-1,4-lactone affording D-oleandronic acid phenylhydrazide which crystallized from MeOH–Et_2O as colourless needles (1.2 mg) mp 133–135°.

Alkaline hydrolysis of compound **3**. Compound **3** (5 mg) was dissolved in 5% methanolic KOH (1 ml) and refluxed for 2 hr. After adding H_2O (0.5 ml), MeOH was removed under red. pres. The aq. concentrate was extracted with $CHCl_3$–MeOH (90:10), dried over Na_2SO_4, filtered and evapd to dryness yielding **4** (2 mg) which crystallized from MeOH–Me_2CO, mp 261–265°, $[\alpha]_D^{25}$ +61.0° (MeOH, *c* 0.1). The TLC, mp, mmp and optical rotation comparison with the authentic material confirmed **4** as sarcostin.

Very mild hydrolysis of compound **7** *with acid.* To a soln of **7** (20 mg) in 80% aq. 1,4-dioxane (3 ml) was added 0.01 N H_2SO_4 (3 ml) and the soln was kept at room temp. After three days, an aliquot (3 ml) of the reaction mixture was taken out and evapd under red. pres. to afford a viscous mass (9.8 mg) which was preparatively separated on a silica gel column giving a chromatographically pure syrupy product (2 mg), $[\alpha]_D^{25}$ +50.4° (H_2O, *c* 0.11). It was found identical to D-cymarose **5** ($[\alpha]_D^{25}$, PC and TLC). From this column an amorphous substance (5 mg) was also obtained which crystallized from Me_2CO–petrol, mp 108–112°, $[\alpha]_D^{25}$ +20.8° (MeOH, *c* 0.12) and identified with pallidine (**1**) ($[\alpha]_D^{25}$, mp, mmp and TLC). The remaining reaction mixture was worked up as above after 92 hr which afforded again a viscous product (10 mg). Preparative separation of the viscous product gave two pure, viscous syrups, **5** (1.6 mg) and **6** (1.5 mg), identified as cymarose and oleandrose, respectively, by comparing with authentic samples. The third pure amorphous product **3** (5 mg) from the column was found to be identical to 12,20-di-*O*-benzoyl sarcostin ($[\alpha]_D$, TLC).

Mono-O-acetyl pallidine (**2**). Compound **1** (10 mg) on acetylation with pyridine (0.5 ml) and Ac_2O (0.4 ml) at 100° for 8 hr afforded **2** as an amorphous residue (10 mg), $[\alpha]_D^{25}$ +10.0°

(MeOH, *c* 0.14). ^{1}H NMR (90 MHz): δ2.05 (3H, *s*, OAc), 4.15 (1H, *m*, H-4′).

Mono-O-acetyl pallidinine (**8**). Compound **7** (10 mg) on acetylation with pyridine (0.5 ml) and Ac_2O (0.4 ml) at 100° for 8 hr afforded **8** as an amorphous residue (9 mg), $[\alpha]_D^{25}$ +46.87° (MeOH, *c* 0.05). ^{1}H NMR (80 MHz): δ7.95–7.3 (10H, aromatic), 5.35 (1H, *m*, H-6) 4.90 (2H, *dd*, H-1′ of cym and ole), 4.80 (1H, *q*, H-20), 4.60 (1H, *dd*, H-12), 4.20 (1H, *m*, H-4′ of cym), 3.75 (2H, *m*, H-5′ of cym and ole), 3.60 (2H, *m*, H-3′ of cym and ole), 3.30 (6H, *s*, 20Me), 3.15 (1H, *m*, H-4′ of ole), 2.35 (2H, *m*, H-2′e of cym and ole), 2.18 (2H, *m*, H-2′a of cym and ole), 2.05 (3H, *s*, OAc), 1.45–1.80 (methylenes of aglycone), 1.05–1.40 (6′-Me of cym and ole, 18-Me, 19-Me, 21-Me).

Acknowledgements—The authors thank Dr. D. K. Kulshreshthra (C.D.R.I., Lucknow) for helpful discussions. One of us (N.K.K.) thanks the C.S.I.R., New Delhi, for the award of a Junior Research Fellowship.

REFERENCES

1. Wada, K., Hayashi, K., Mitsuhashi, H. and Bando, H. (1982) *Chem. Pharm. Bull.* **30**, 3500.
2. Reichstein, T. (1967) *Naturwissenschaften* **54**, 53.
3. Barton, G. M., Evans, R. S. and Gardner, J. A. F. (1952) *Nature* **170**, 249.
4. Tschesche, R., Grimmer, G. and Seehofer, F. (1953) *Chem. Ber.* **86**, 1235.
5. Nagata, W., Tamm, C. and Reichstein, T. (1957) *Helv. Chim. Acta* **40**, 41.
6. Shaub, F., Kaufman, H. Stöcklin, W. and Reichstein, T. (1968) *Helv. Chim. Acta* **51**, 738.
7. Rangaswami, S. and Reichstein, T. (1949) *Helv. Chim. Acta* **32**, 939.
8. Blindenbacher, F. and Reichstein, T. (1948) *Helv. Chim. Acta* **31**, 2061.
9. Renkonen, O., Schindler, O. and Reichstein, T. (1959) *Helv. Chim. Acta* **42**, 182.
10. Jaeggi, K. A., Weiss, E., Wehrli, W. and Reichstein, T. (1967) *Helv. Chim. Acta* **50**, 1201.
11. Brown, P., Bruschweiler, F., Petit, G. R. and Reichstein, T. (1971) *Org. Mass. Spectrom.* **5**, 573.
12. Allgeier, H. (1968) *Helv. Chim. Acta* **51**, 311.
13. Krasso, A. F., Weiss, E. and Reichstein, T. (1963) *Helv. Chim. Acta* **46**, 1691.
14. McLennan, A. P., Randall, H. M. and Smith, D. W. (1963) *Analyt. Chem.* **31**, 2020.