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# TWO PYRAN TYPE GLYCOSIDES FROM *SAPONARIA* AND *DIANTHUS*

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**Key Word Index**—*Saponaria officinalis*; *Dianthus barbatus*; *D. deltoides*; Caryophyllaceae; sapopyroside; barbapyroside; 2,3-dihydro 4-*O*-β-D-glucopyranosyl 3-hydroxy-2-methyl-4H-pyran.

**Abstract**—2,3-Dihydro 4-*O*-β-D-glucopyranosyl 3-hydroxy-2-methyl-4H-pyran was isolated from *Saponaria officinalis* and its relative configuration and conformation determined. A new glycoside, isomeric with the former but having a different configuration at C-4, has been isolated from *Dianthus barbatus* and *D. deltoides*.

## INTRODUCTION

When extracting D-pinitol from Caryophyllaceaous plants, one of us obtained in 1954 and 1956 two new glycosides, one from *Saponaria officinalis* and the other from *Dianthus barbatus* and *D. deltoides* (carnations). Their description could not be published on account of insufficient structural data. The present paper adds additional information to a preliminary report [1] giving elucidation of structure and configuration of these compounds on the basis of their physical properties and spectral data.

## RESULTS AND DISCUSSION

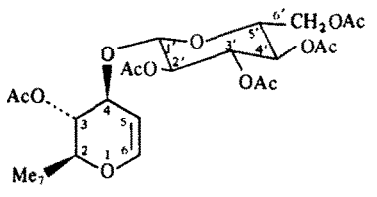
The glycoside extracted from *S. officinalis* was named sapopyroside. Its structure was elucidated by means of its mass spectrum and $^1$H NMR spectrum performed on its penta-acetate (**1**). This compound is 2,3-dihydro-3,4-dihydroxy-2-methyl-4H-pyran substituted at the 4-position by β-glucopyranose. It was identified on the basis of literature data with the glycoside obtained by Shimizu *et al.* [2] from the *D. superbus* var. *longicalycinus*. The configuration of their compound is lacking in their report.

Concerning sapopyroside acetate, the sequence of protons was established by specific irradiations. The $^1$H NMR spectrum revealed a double bond located at C-5–C-6 and a methyl at the 2-position. That the glucopyranose is substituted at the 4-position can be deduced from the known ether shielding effect as compared with the acetate one. In the case of 2,3-dihydro-3,4-dihydroxy-2-methyl-4H-pyran diacetate, Shimizu *et al.* [2] give δ 5.00 for the H-3 chemical shift and δ 5.30 for the H-4 chemical shift; in our compounds, the values obtained are 5.08 and 5.26 for H-3, 4.17 and 4.57 for H-4.

The aglycone configuration was established by NOE measurements; the enhancement percentage of signal amplitudes are shown in Fig. 1. NOE proved on one hand that the three H-7s are spatially near H-3, and on the other hand that H-3 was quite remote from H-2 and H-4. These results establish the relative stereochemistry at C-2, C-3 and C-4: the three substituents having a pseudo equatorial configuration (**1**).

Some authors interpret the small coupling constants for $J_{2ax,3ax}$ and $J_{3ax,4ax}$ in terms of an interconversion equilibrium between two conformations. The pyran aglycone spectrum at −80° does not show any significant change but we think that these values may be due to the flattening of the cycle produced by the double bond and the bulky substituent at the 4-position. In agreement with this proposal, a very small value was noticed for $J_{3,5}$ coupling which is a sensitive indicator of this conformational change [3].

The glycoside extracted from *D. barbatus* and *D. deltoides* was named barbapyroside (penta-acetate **2**). It is a new compound being an isomer of sapopyroside. In barbapyroside acetate, the H-3–H-7 distance is much greater than that in sapopyroside acetate but H-3 is nearer to H-2 and H-4 (**2**). Furthermore, $J_{2,3}$ is lower than that in sapopyroside acetate one ($J_{ax,eq}$ instead of $J_{ax,ax}$). These results and the occurrence of long range coupling constants $J_{2ax,4ax}$ and $J_{3eq,5eq}$ establish the relative stereochem-




**1**

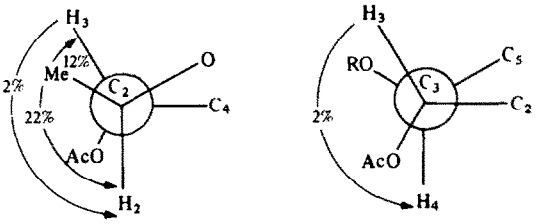
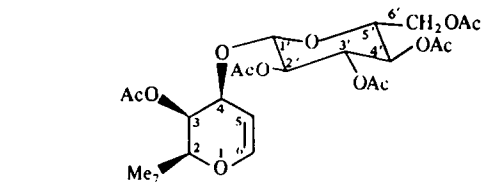



Fig. 1. Configuration of the aglycone of sapopyroside as determined from nuclear Overhauser experiments.



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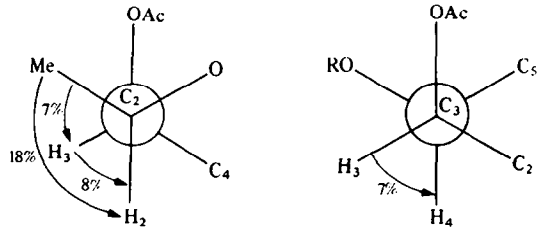

Fig. 2. Configuration of the aglycone of barbapyroside as determined from nuclear Overhauser experiments.

istry at C-2, C-3 and C-4. The 2-methyl and the 4-β-D-glucopyranosyl being equatorial whereas the 3-acetoxyl is axial (2).

These two glycosides are included in a group of 16 theoretical isomers: the glucose can be fixed at the 3- or 4-position of eight isomer aglycones differing by the stereochemistry of their three asymmetrical carbon atoms. The pyran aglycones have a structural similarity to maltol (3-hydroxy 2-methyl 4H-pyran-4-one). The theoretical transformation to maltol only requires elimination of H-2 and H-3, thus producing a double bond, and simultaneous oxidation of the 4-CHOH to C=O.

Dianthoside, the β-D-glucopyranoside of maltol, is widely distributed in *Saponaria* and *Dianthus* genera. A chromatographic method used for investigations on the distribution of this compound (red spots) also reveals the pyran glycosides (blue spots). It was also applied to a number of Caryophyllaceae-Silenoideae. Occurrence of pyran glycosides was only found in *S. cerastoides*, *S. orientalis*, *Dianthus caryophyllus* (one specimen out of three), *D. chinensis* and *D. noeanus* (one specimen out of three). On the basis of $R_f$ values before and after emulsin hydrolysis, the occurrence of sapopyroside is possible in *S. orientalis*, but *S. cerastoides* and *D. chinensis* contain glycosides differing from the two compounds described above.

Chromatographic investigations were also performed on a number of Pinaceae and species belonging to several families of dicotyledons where maltol was previously reported [4]. No blue spot could be detected. Consequently, until now, it appears that pyran glycosides are scarcely distributed in plants.

## EXPERIMENTAL

*Sapopyroside.* Air dried and powdered leaves of *S. officinalis* var. *fl. pleno* harvested in May were extracted in a Soxhlet apparatus with $Me_2CO$. The voluminous deposit which appears in $Me_2CO$ was washed with $Me_2CO$, $Et_2O$ and EtOH to provide crude glycoside; yield 11% dry wt. In July, only 3.5% was obtained. In September and November, the deposit was syrupy and required lead acetate treatment for isolation of the glycoside. The glycoside was also extracted from stems or flowers (1.1 or 0.45% crude extract) but was not found in the roots. Purification was achieved by crystallizations from EtOH and EtOAc. Anhydrous colourless prisms or needles $C_{12}H_{20}O_8$, mp 179° $[\alpha]_D$ −64° ($H_2O$; *c* 1%). Colour reactions, $H_2SO_4$: red brown turning slowly to blue black with ppt; HCl: yellowish turning to green; NaOH, $FeCl_3$: no colouration. Hydrolysis ($H_2SO_4$, 2%) at 100°: the soln turns yellow and produces a reddish ppt. The aglycone is labile but a small amount was obtained in crystalline form after enzymatic hydrolysis by almond emulsin. $H_2O$ soluble, mp 70°. $C_6H_{10}O_3$. Glucose identified by PC.

*Acetylation.* $Ac_2O$–pyridine, overnight at room temp. Penta-acetate: colourless needles, mp 136°. Violet with $H_2SO_4$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1750, 1740 (>C=O), 1655 (>C=C<), 1375, 1240, 1210, 1055, 1030, 900. DICMS (probe) 100 eV, *m/z* (rel. int.): 520 $[M+NH_4^+]^+$ (41), 503 $[M+H]^+$ (0,3), 331 (61), 271 (14), 169 (74), 155 (100), 139 (10), 127 (10), 109 (75). $^1$H NMR (500, 13 MHz, $CDCl_3$): δ6.42 (1H, *dd*, $J_{5,6}$ = 6.2 Hz, $J_{4,6}$ = 1.0 Hz, H-6), 5.20 (1H, *dd*, $J_{2',3'}$ = 9.5 Hz, $J_{3',4'}$ = 9.5 Hz, H-3'), 5.08 (1H, *dd*, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 4.7 Hz, $J_{3,5}$ = 0.7 Hz, H-3), 5.08 (1H, *dd*, $J_{4',5'}$ = 9.8 Hz, H-4'), 4.95 (1H, *dd*, $J_{1',2'}$ = 7.9 Hz, H-2'), 4.78 (1H, *ddd*, $J_{4,5}$ = 3.6 Hz, H-5), 4.69 (1H, *d*, H-1'), 4.21 (2H, *ddd*, $J_{5',6'a}$ = 2.5 Hz, $J_{5',6'b}$ = 4.5 Hz, $J_{6'a,6'b}$ = 12.2 Hz, $CH_2$-6'), 4.17 (1H, *dq*, $J_{2,7}$) = 7.0 Hz, H-2), 4.17 (1H, *ddd*, H-4), 3.72 (1H, *ddd*, H-5'), 2.08 (3H, *s*, AcO), 2.07 (3H, *s*, AcO), 2.02 (3H, *s*, AcO), 2.01 (3H, *s*, AcO), 1.99 (3H, *s*, AcO), 1.29 (3H, *d*, Me-7).

*Barbapyroside.* Leaves of *D. barbatus* harvested in May–June: crude product from $Me_2CO$ 6–8% dry wt. Stems and flowers: rather impure product 2–3% dry wt. Roots: not present. Aerial parts of *D. deltoides*, in October: reddish brown deposit from $Me_2CO$ 5–6% dry wt; basic lead acetate treatment afforded a yellowish crystalline compound. Purification as for sapopyroside. Anhydrous colourless prisms or needles, $mp_{slow}$ 215°, $mp_{inst}$ 230°; $[\alpha]_D$ −24° ($H_2O$; *c* 1%). Less soluble than sapopyroside in usual solvents; coloured reactions and other properties very similar to sapopyroside. The aglycone is labile: obtained in amorphous form after enzymatic hydrolysis.

*Acetylation* afforded a penta-acetate, mp 145–146°. IR $\nu_{max}$ $cm^{-1}$: 1750 (>C=O), 1645 (>C=C<), 1370, 1230, 1035, 905. MS *m/z* (rel. int.): 520 $[M+NH_4^+]^+$ (21), 503 $[M+H]^+$ (0.3), 331 (71), 271 (23), 163 (60), 155 (10), 139 (17), 127 (25), 109 (72). $^1$H NMR: δ6.40 (1H, *dd*, $J_{4,6}$ = 1.6 Hz, $J_{5,6}$ = 6.4 Hz, H-6), 5.26 (1H, *ddd*, $J_{2,3}$ = 1.0 Hz, $J_{3,4}$ = 4.8 Hz, $J_{3,5}$ = 1.8 Hz, H-3), 5.19 (1H, *dd*, $J_{2',3'}$ = 9.5 Hz, $J_{3',4'}$ = 9.3 Hz, H-3'), 5.07 (1H, *dd*, $J_{4',5'}$ = 9.7 Hz, H-4'), 4.98 (1H, $J_{1',2'}$ = 7.9 Hz, H-2'), 4.71 (1H, *ddd*, $J_{4,5}$ = 2.0 Hz, H-5), 4.64 (1H, *d*, H-1'), 4.57 (1H, *ddd*, $J_{2,4}$ = 0.9 Hz, H-4), 4.20 (1H, *ddq*, $J_{2,7}$ = 6.7 Hz, H-2), 4.20 (2H, $J_{5',6'a}$ = 2.3 Hz, $J_{5',6'b}$ = 4.2 Hz, $J_{6'a,6'b}$ = 12.3 Hz, $CH_2$-6'), 3.67 (1H, *ddd*, H-5'), 2.12 (3H, *s*, AcO), 2.09 (3H, *s*, AcO), 2.04 (3H, *s*, AcO), 2.02 (3H, *s*, AcO), 2.00 (3H, *s*, AcO), 1.23 (3H, *d*, Me-7).

*Chromatography.* Aq. extracts of plants were extracted with warm EtOH and drops deposited on silica gel 'Polygram SIL G/UV$_{254}$', elution solvent EtOH–*n*-BuOH–$H_2O$ (40:11:19). Visualization UV, spraying with $FeCl_3$ or methanolic $H_2SO_4$ and heating to 80° during several min. Spots are blue with $FeCl_3$, greyish blue with $H_2SO_4$. $R_f$ values: sapopyroside 0.61, aglycone 0.73; barbapyroside 0.45, aglycone 0.57.

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# ANTIBACTERIAL CONSTITUENTS OF THE RED ALGA *CYSTOCLONIUM PURPUREUM*

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**Key Word Index**—*Cystoclonium purpureum*; Rhodophyllidacea; α-carotene; plastoquinone-9; *trans*-phytol; ubiquinol-9; lutein; fucoxanthin; polysaturated fatty acids; antibacterial acids.

**Abstract**—The red alga *Cystoclonium purpureum* was found to contain α-carotene, plastoquinone-9, *trans*-phytol, ubiquinol-9, lutein and fucoxanthin. The composition of the antibacterial fatty acid fraction was determined by GC/MS.

## INTRODUCTION

An extract of the previously uninvestigated red alga *Cystoclonium purpureum* exhibited strong antibacterial activity. We now report our findings on its chemical constituents.

## RESULTS AND DISCUSSION

*Cystoclonium purpureum*, collected at Lepreau Ledges, New Brunswick, was extracted with methanol in a Soxhlet apparatus and the chloroform soluble portion of the evaporated methanol extract subjected to silica gel CC.

Following elution of free sterols, a fatty acid fraction was obtained which showed significant antibacterial activity in disc diffusion assays. This mixture was fractionated by reversed phase prep. TLC and three fractions (F1, F2 and F3) displayed significant antibacterial activity (Table 1) while the others (F4, F5 and F6) did not. The fatty acid fractions on treatment with ethereal diazomethane provided the corresponding methyl esters which were analysed by GC/MS, the results of which are summarized in Table 2. Examination of the six acid fractions revealed that they were, for the most part, complex mixtures with considerable overlap of components.

In a recent report [1] on the antibacterial acids from the diatom *Navicula delognei* we have noted the presence and antibiotic activity of (6*Z*,9*Z*,12*Z*,15*Z*)-hexadecatetraenoic and an ester of the known antibiotic (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic acid. Present indications are that several *cis* polyunsaturated acids are responsible for the antibacterial activity of the *C. purpureum* extract.

## EXPERIMENTAL

*General.* $^1$H NMR spectra were recorded at 200 MHz using TMS as int. standard. An HPLC unit equipped with an absorbance and a refractive index detector, employing a μ-porasil column was used for HPLC purification. Analytical and prep. TLC were performed with precoated silica gel G (Kieselgel 60, F-254) and reverse phase ($KC_{18}F$) plates. GC/MS analysis was carried out on a quadrupole EI-CI system at 70 eV, employing an SP-2330 (30 m) column at 40–160° (10°/min), 160–200° (5°/min) and 14 psi.

Fresh *C. purpureum* Batters (2.3 kg wet wt), collected at Lepreau Ledges, New Brunswick (May 1983), was finely chopped and immersed in MeOH for 2 hr and then extracted in a Soxhlet for 48 hr. The extract was coned under red. pres. and the residue (19 g) dissolved in $H_2O$ (250 ml) and extracted with $CHCl_3$ (3 × 250 ml). Removal of $CHCl_3$ yielded a dark green solid (12.5 g), which was subjected to CC on silica gel G (250 g) eluting with hexane, hexane–EtOAc, $CHCl_3$ and $CHCl_3$–MeOH mixtures in sequence. Fractions were monitored by TLC, combined and purified by prep. TLC and HPLC. In order of elution, the following compounds were obtained and identified by comparison of their spectral and physical characteristics with literature data: α-carotene [2] (0.069 g), plastoquinone-9 [3, 4] (0.044 g, purified by prep. TLC and HPLC with 3% EtOAc in hexane), *trans*-phytol [5] (0.108 g), and ubiquinol-9 [6–8] (0.021 g, purified by HPLC with 1% MeOH in $CHCl_3$). Further elution provided free sterols (0.755 g), fatty acids (0.828 g,