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# A SPIROSTANOL GLYCOSIDE FROM THE RHIZOMES OF *OPHIOPOGON INTERMEDIUS*

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**Key Word Index**—*Ophiopogon intermedius*; Haemodoraceae; rhizomes; spirostanol glycoside; ruscogenin; spermicidal potential.

**Abstract**—A new ruscogenin glycoside has been characterized from the rhizomes of *Ophiopogon intermedius*.

## INTRODUCTION

Saponins have been described to have spermicidal potential [1]. Ruscogenin glycosides have been reported from *Ophiopogon* sp. [2, 3]. We have reported a few known compounds from the rhizomes of *O. intermedius* [4] and this communication deals with the characterization of a new ruscogenin glycoside.

## RESULTS AND DISCUSSION

Saponin **1**, a (25*R*)-spirostanol derivative (IR) was found to have $M_r$ of 724 as indicated by the protonated molecular ion at *m/z* 725 in its FDMS. A peak at *m/z* 593 $[M+H-132]^+$ arose by loss of a terminal pentose moiety whereas a peak at *m/z* 430 was suggestive of a dihydroxy spirostene nucleus.

Acidic hydrolysis of **1** gave a genin, identified as ruscogenin, and D-glucose and L-arabinose. The permethylate **1a**, prepared by Kuhn's method, on methanolysis gave another aglycone, identified as the 3-*O*-methylether of ruscogenin by comparison of physical constants [5]. The sugar portion was hydrolysed to give 2,3,4-tri-*O*-methyl-L-arabinose and Wallenfel's positive [6] 3,4,6-tri-*O*-methyl-D-glucose. The anomeric linkages were deduced as $\beta$- for D-glucose and $\alpha$- for L-arabinose by the application of Klyne's rule [7] and examination of the $^1$H NMR spectrum.

Thus, **1** was identified as ruscogenin 1-*O*-[$\alpha$-L-arabinopyranosyl (1→2)]-$\beta$-D-glucopyranoside. A 1.5% (w/v) solution caused total immobilization of human spermatozoa when tested by published methods [8, 9].

## EXPERIMENTAL

Mps: uncorr. IR recorded in SP-3-200. CC over silica gel (BDH, 100–120 mesh) and TLC on Kieselgel 60G (Merck). The spots on TLC were visualized by spraying with 10% $H_2SO_4$ and on prep. TLC by $H_2O$. PC was performed on Whatman no. 1 paper by the descending mode using aniline hydrogen phthalate as visualizer. The following solvent systems were used: (A) $CHCl_3$–MeOH (4:1), (B) $C_6H_6$–EtOAc (4:1), (C) $C_6H_6$–$Me_2CO$ (4:1), (D) EtOAc–pyridine–$H_2O$ (10:4:3) and (E) *n*-BuOH–EtOH–$H_2O$ (5:1:4).

*Isolation of compound* **1**. Cut rhizome material (10 kg) was extracted with MeOH (25 l) and the solvent was removed *in vacuo*. The extract (600 g) was partitioned between $Et_2O$ and $H_2O$, then the $H_2O$ layer was extracted with *n*-BuOH. The butanolic residue was fractionated by CC (solvent A) to afford glycosides [4] and two compounds **1** and **2** (120 and 200 mg, respectively).

*Compound* **1**. Colourless crystals (120 mg) from MeOH, mp: 203–205° (dec.), $[\alpha]_D^{20}$ −71° ($CHCl_3$–MeOH; *c* 1.0); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3500-3300 (OH), 985, 920, 900, 865 (intensity 900 > 920, 25*R*-spiroketal). $^1$H NMR (pyridine): $\delta$ 0.73 (3H, *br d*, –CH–Me), 0.94 (3H, *s*, Me), 1.14 (3H, *d*, *J* = 6 Hz, –CH–Me), 1.29 (3H, *s*, Me), 4.91 (1H, *d*, *J* = 6 Hz, H-1 of ara), 4.98 (1H, *d*, *J* = 7 Hz, H-1 of glu). (Found: C, 60.92; H, 8.5. $C_{38}H_{60}O_{13}$ requires C, 61.6; H, 8.4%).

*Acidic hydrolysis of* **1**. Compound **1** (50 mg) in 2 M HCl–MeOH (1:1, 10 ml) was refluxed for 3 hr and the aglycone was then purified by prep. TLC (solvent B), mp 205–208°, $[\alpha]_D^{20}$ −110° ($CHCl_3$–MeOH; *c*, 0.5); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (OH), 982, 920, 900, 865 (intensity 900 > 920) (lit. [2] mp 205–207°, $[\alpha]_D^{18}$ −112.4°, pyridine; *c* 0.31). The neutralized and conc. aq. hydrolysate contained D-glucose and L-arabinose (PC, solvent D, authentic samples run in parallel). Methylation of **1** (50 mg) in MeI (4 ml) and $Ag_2O$ (300 mg) in DMF (1 ml) followed by prep. TLC (solvent C) afforded **1a** (40 mg, no OH in IR).

*Methanolysis and hydrolysis of* **1a**. Compound **1a** (35 mg) in dry 10% HCl–MeOH (10 ml) was refluxed (3 hr), the solvent was removed and the aglycone (12 mg) was purified by prep. TLC (solvent C). Colourless needles, mp 222–224°, IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (OH), 1250, 982, 920, 900, 865; identified as ruscogenin 3-*O*-methylether (**5**). Compound **1a** (10 mg) was hydrolysed with 2 M HCl–MeOH (5 ml) for 3 hr and the concd. aq. hydrolysate was subjected to PC (solvent E) to identify 2,3,4-tri-*O*-methyl-L-arabinose and 3,4,6-tri-*O*-methyl-D-glucose ($R_G$ values 0.95 and 0.84, respectively).

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## REFERENCES

1. Kamboj, V. P. and Dhawan, B. N. (1982) *J. Ethnopharmacol.* **6**, 191.

2. Watanabe, Y., Sanada, S., Ida, Y. and Shoji, J. (1983) *Chem. Pharm. Bull.* **31**, 1980.
3. Watanabe, Y., Sanada, S., Ida, Y. and Shoji, J. (1984) *Chem. Pharm. Bull.* **32**, 3994.
4. Rawat, M. S. M., Negi, D. S., Pant, G. and Panwar, M. S. (1988) *Pharmazie* (in press).
5. Watanabe, Y., Sanada, S., Ida, Y. and Shoji, J. (1983) *Chem. Pharm. Bull.* **31**, 3486.
6. Wallenfel, K. (1950) *Naturwissenschaften* **37**, 491.
7. Klyne, W. (1950) *Biochem. J.* **47**, 41.
8. Banerji, R., Misra, G. and Nigam, S. K. (1985) *Fitoterapia* **LVI**, 186.
9. Blackshaw, A. W. and Emmens, C. W. (1951) *J. Physiol.* **114**, 16.

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# (*E*)-*O*-*p*-COUMAROYL-, (*E*)-*O*-FERULOYL-DERIVATIVES OF GLUCARIC ACID IN CITRUS

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**Key Word Index**—*Citrus sinensis*; Rutaceae; *p*-coumaroylglucaric acid, feruloyl- and diferuloylglucaric acid.

**Abstract**—2-(*E*)-*O*-*p*-Coumaroyl-, 2-(*E*)-*O*-feruloyl- and 2,4-(*E*,*E*)-*O*-diferuloylaldaric acid esters from orange peel have been isolated and identified spectroscopically. GC-analysis of the TMS-derivatives of the hydrolysis products indicated the aldaric acid was glucaric acid.

## INTRODUCTION

In a previous study of the hydroxycinnamic acid derivatives in citrus, we reported the isolation and identification of 2-(*E*)-*O*-*p*-coumaroyl- and 2-(*E*)-*O*-feruloylgalactaric acid from orange peel [1]. The esters were shown by HPLC to be two compounds of a group of several unidentified *p*-coumaric and ferulic acid derivatives. Here we present evidence of the identity of more of these closely related compounds. Esters of hexaric acid moieties with a hydroxycinnamic acid have been found in the leaves of various solanaceous plants [2, 3] and in rye [4].

## RESULTS AND DISCUSSION

The fractions studied were obtained by preparative HPLC. In the case of the monoesters several narrow eluting fractions were isolated which gave highly hygroscopic white crystalline substances upon freeze drying. The purity and stability of each isolated fraction was established by analytical HPLC. This was also used to check for rearrangements during the isolation and purification procedures.

The ferulic and *p*-coumaric acid fractions each showed a pattern of four peaks by analytical HPLC. These could be unambiguously differentiated from the previously identified *p*-coumaroyl- and feruloylgalactaric acid peaks which had similar retention times. Hydrolysis of each fraction gave the free hydroxycinnamic acid. GC-analysis of the TMS-derivatives of the compounds produced by enzymatic hydrolysis indicated glucaric acid. A clear distinction between glucaric acid, galactaric acid and their lactones was possible. One peak from each fraction was isolated in amounts sufficient for NMR- and MS-analysis. The nature of the various moieties in the molecules were unambiguously identified from the cross peaks in the 2D $^1$H COSY spectra (**1**) or by homodecoupling (**2**). The low field shift of H-2 of the aldaric acid moiety was a clear indication of the point of attachment of the hydroxycinnamic acid substituent. As the absolute geometry of this asymmetric centre is not determinable by NMR the unambiguous identity of the glucaric acid derivatives cannot be established. This would only be possible by X-ray analysis of a suitable derivative. Thus the compounds are either 2- or 5-substituted glucaric acids.

The *p*-coumaroylglucaric acid is one of two main components which could not be separated by HPLC. From the FABMS the second compound has a $M_r$ 14 mass units higher with a methoxyl group replacing one of the hydroxyl groups in the glucaric acid moiety. The feruloylglucaric acid ester showed a small additional component, which is probably the benzoylglucaric acid from the FABMS. The other peaks in the two fractions could not be obtained in a form suitable for NMR and MS studies. However, it is quite probable that these correspond to the other open chain isomeric forms as positional isomerism is a well-known phenomenon which occurs with esters of phenolic acids with polyhydroxy compounds [2]. Lactone forms were not present, as an orange extract showed the same pattern of peaks before and after treatment with alkali.

The purified diferulic compound was a white crystalline substance that was less hygroscopic than the monoes-