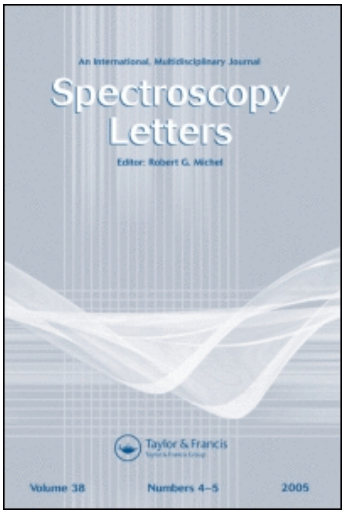

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Two-Dimensional NMR Identification of an Acetophenone Glycoside Isolated from Solanaceous Cell Suspensions

D. Marty[a]; F. Gillet-Manceau[b]; M. A. Fliniaux[b]; A. Jacquin-Dubreuil[b]; J. P. Monti[a]
[a] Laboratoire de Biophysique, Faculte de Pharmacie, Amiens Cedex, France [b] Laboratoire de Pharmacognosie et Phytotechnologie, Faculte de Pharmacie, Amiens Cedex, France

To cite this Article Marty, D. , Gillet-Manceau, F. , Fliniaux, M. A. , Jacquin-Dubreuil, A. and Monti, J. P.(1996) 'Two-Dimensional NMR Identification of an Acetophenone Glycoside Isolated from Solanaceous Cell Suspensions', Spectroscopy Letters, 29: 1, 169 — 174
To link to this Article: DOI: 10.1080/00387019608001592
URL: http://dx.doi.org/10.1080/00387019608001592

SPECTROSCOPY LETTERS, 29(1), 169-174 (1996)

TWO-DIMENSIONAL NMR IDENTIFICATION OF AN ACETOPHENONE GLYCOSIDE ISOLATED FROM SOLANACEOUS CELL SUSPENSIONS

Key Words : Two-dimensional NMR, homonuclear chemical shift correlation, acetophenone glycoside, solanaceous cells.

D. Marty[1], F. Gillet-Manceau[2], M.A. Fliniaux[2], A. Jacquin-Dubreuil[2], J.P. Monti[1,3]

[1]Laboratoire de Biophysique
[2]Laboratoire de Pharmacognosie et Phytotechnologie
Faculté de Pharmacie, 1 rue des Louvels 80037, Amiens Cedex, France.
[3]Address for correspondence

ABSTRACT

From a solanaceous cell suspension an acetophenone glycoside, androsin, has been isolated along with one polyphenol glycoside, scopolin. The androsin presence is described for the first time in solanaceous cell suspension.

INTRODUCTION

Many plant cell cultures are known to biosynthetize and accumulate polyphenols. Coumarin compounds were reported to be present in Solanaceous, and mainly tobacco cell cultures. In a previous work (1), we found that *Duboisia myoporoides* (Solanaceae) cell suspensions accumulated scopolin and also an another compound. In the present work, we identify this molecule as androsin, a

monoglycoside of acetovanillon, on the basis of 1D and 2D NMR spectral data and MS results. The occurence of this compound has been reported until now in some apocynaceous (2) and scrophulariaceous plants (3,4). Its presence in a solanaceous plant cell culture is an original result.

RESULTS AND DISCUSSION

^{1}H-NMR and ^{13}C-NMR results (Table 1) show the presence of a glucosyl moiety and the H-1' anomeric proton coupling value is consistent with values for ß-anomer (7-10 Hz) but not α-anomer (2-4 Hz) (6). The ß-anomer is confirmed by the C-1' chemical shift value (7). In the same manner for aglycone moiety, ^{1}H-NMR coupling features show a substituted aromatic ring with three substitutents (carboxyl, methoxyl and glycosyl groups) at C-1, C-3 and C-4. The substitutent positions are obtained by NOESY experiments with cross peak correlations H-1'/ H-5 then H-6/ $COCH_3$, H-2/ $COCH_3$ and H-2/ OCH_3 (figure 1). ^{13}C-NMR confirm this structure with chemical shift values in good agreement with those of literature although the solvent used (pyridine-d5) is different (4).

For MS characteristic fragment ions were : EIMS m/z (%) : 511 (2.1), 361 (100), 238 (60.0), 217 (82.5) and CIMS m/z (%) : 601 (2.0), 511 (5.1), 451 (2.9), 361 (100), 167 (9.0), 239 (41.1). CIMS analysis shows the fragment ions m/z 601 $(M-15)^+$ classicaly recovered after the loss of a methyl group and m/z 511 (m/z 601-TMSOH) (8). We observe the glycosyl moiety m/z 451 with his fragments m/z 361 (m/z 451-TMSOH) and m/z 217 for EI common to all silylated carbohydrates (8). Lastly for the aglycone moiety, we have the fragment ions m/z 167 $(aglycone+H)^+$. In EIMS, the fragment ion m/z 238 originates in TMS-aglycone is obtained after a classical transfer of the TMS group (8,9).

In order to confirm this structure and to analyse more precisely the glycosyl moiety, acetovanillon was added in the culture media. After glucosylation by plant cell suspensions, the compound obtained, studied by NMR, has shown a similar structure.

Table I : Proton and ^{13}C chemical shift (ppm) assignments of androsin in D_2O

	^{13}C	^{1}H
1	132.1	
2	112.2	7.22 (d; 1.9)
3	149.0*	
4	150.8*	
5	115.2	6.95 (d; 8.7)
6	124.7	7.38 (dd; 8.7, 1.9)
CO	202.8	
$CO-CH_3$	26.6	2.39 (s)
OCH_3	56.6	3.70 (s)
1'	100.4	5.02 (d; 7.4)
2'	73.4	
3'	77.0	
4'	70.0	3.47-3.27 (m; 4H)
5'	76.2	
6'	61.2	3.71 (dd; -12.4, 2.1)
		3.54 (dd; -12.4, 5.4)

* Assignments may be reversed.

The coupling constants (J values in Hz) are shown in the parentheses.

From these data the compound isolated is androsin, the 4-ß-D *glucopyranoside of acetovanillon.*

EXPERIMENTAL

The product accumulated in the plant cell cultures was extracted by 70% (v/v) methanol under reflux. The extract was concentrated to dryness, under reduced pressure. The residue was resuspended in absolute ethanol. The suspension was then filtered. The filtrate was concentrated, and the residue was dissolved in the eluent used in the next step that was the chromatographical

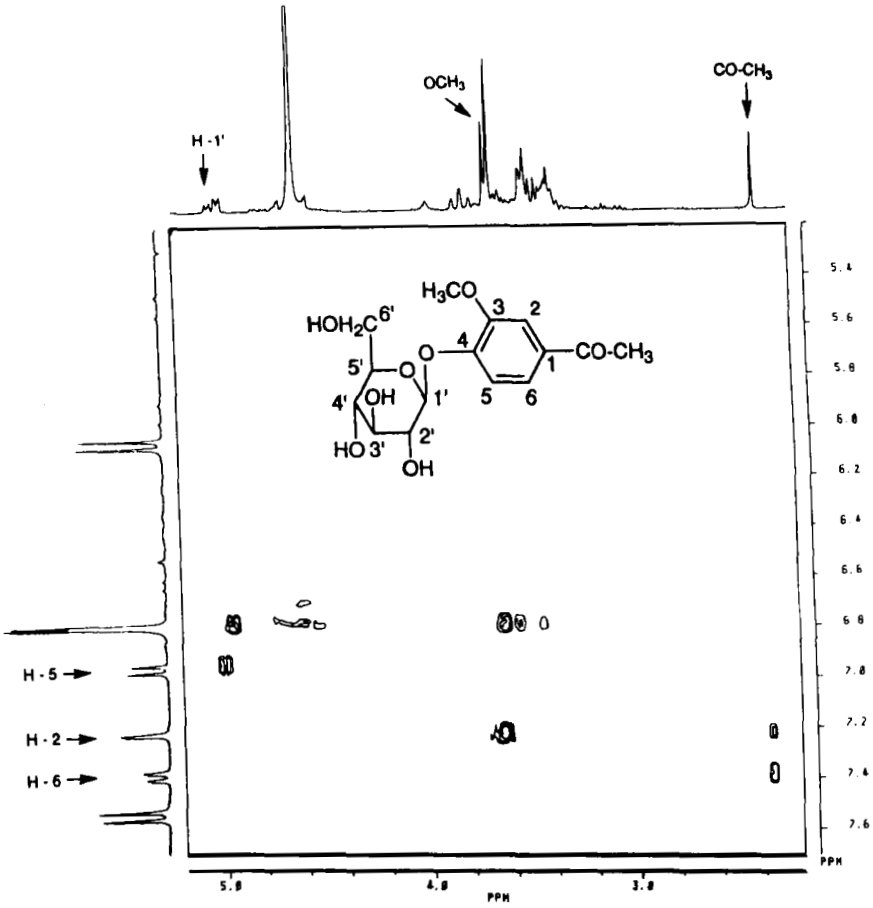

Fig. 1 : Noesy spectrum of mixture 4-ß-D glucopyranoside of acetovanillon (androsin) and scopolin. Acquisition : spectral widths F_2 = 1650 Hz and F_1 = ±825 Hz, allowing a digital resolution of 3.2 Hz per point with 256 (t_1) x 1024 (t_2) points; mixing time = 750 ms (random variation used 30 ms). Processing : zero filled to 512 x 1024; sine-bell apodization (both t_1 and t_2) and symmetrization of the final data matrix. Expansion of spectral region : 5.2-7.7 ppm (F_1) / 2.2-5.2 ppm (F_2). Assignments of hydrogens spatially proximal are illustrated for androsin.

isolation of the product. Semi preparative reversed-phase HPLC was used for this last step of the purification of androsin : the column was a C18 column (25 x 1 cm), particle size : 10 μm; the eluent was an acetonitrile (12,5%)-methanol (12,5%) aqueous solution. The flow rate was 3 ml/min. Issued from the column, the eluent went through the flow cell of a Perkin-Elmer UV-Vis spectrophotometer, where the D.O. was measured.at 285 nm. The elution profile was recorded using a tracelab recorder (OSI France).

Mass spectrometry : after trimethylsilyl derivatisation (5) , the spectra were recorded with a Hewlett-Packard 5987 gas chromatograph-mass spectrometer. The experimental conditions were : capillary column WSCOT (12 m x 0.2 mm id) with methylsilicone (Hewlett-Packard) as stationary phase; carrier gas, helium (column head pressure 1 bar); temperature program, 60 to 270 °C at 14 °C/min; ion-source temperature, 200 °C; injector operating with splitless mode (0.5 min splitless period); emission current, 300 mA; electron energy, 70 eV for electron impact (EIMS) and 130 eV for chemical ionization (CIMS); reagent gas was methane and ion source pressure was 1 torr for CIMS.

NMR spectroscopy : ^{1}H and ^{13}C spectra were recorded at 300.13 and 75.47 MHz respectively in D_2O with a Bruker AM 300 spectrometer. For ^{1}H-NMR, HOD signal at 4.6 ppm was used as internal reference and for ^{13}C-NMR resonance chemical shifts were relative to sodium formate at 172.0 ppm. Proton and carbon resonance assignments and structural elucidation were performed by COSY, NOESY and DEPT experiments.

Resonance multiplicities for ^{13}C were established via the acquisition of DEPT spectra obtained for proton pulses $P_\theta = 135°$ (CH and CH_3 differentiated from CH_2). The $(2J)^{-1}$ delay was set equal to 3.7 ms.

The homonuclear ^{1}H -^{1}H chemical shift correlated two-dimensional diagram was obtained using the COSY-90 pulse sequence. The spectral widths were F_2 = 1650 Hz and $F_1 = \pm$ 825 Hz, allowing a digital resolution of 3.2 Hz per point with 256 (t_1) x 1024 (t_2) points. After zero filling to 512 x 1024, sine-bell apodization (both t_1 and t_2) was applied, followed by symmetrization of the final

data matrix. The relaxation delay was 2s. For NOESY, the experimental conditions are in the Figure 1 legend.

ACKNOWLEDGEMENTS

We thank Dr C. Aubert for recording mass spectra and for his helpful discussions.

REFERENCES

1-Fliniaux M.A., Gillet-Manceau F., Marty D., Macek T., Monti J.P., Jacquin-Dubreuil A. Ability of some solanaceous and Papaver cell suspensions to produce and glucosylate scopoletin and esculin. Submitted

2-Paris R.R., Moyse H. *Précis de Matière Médicale*, ed. Paris: Masson, 1971

3-Stuppner H., Wagner H. Minor Iridoid and Phenol Glycosides of *Picrorhiza kurrooa.* Planta Med. 1989; 55: 467-469.

4-Junior P. Acetophenonglucoside aus *Penstemon pinifolius.* Planta Med. 1986; 52: 218-220.

5-Larsen G.L., Bakke J.E. Mass spectral characterization of the glucuronide conjugates of terbutryne {2-(t-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazinic} metabolites from rats and goat. Biomed. Mass Spectrom. 1978; 5: 391-394.

6-Jackman L.M. and Sternhell S. Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, ed. New York: Pergamon Press, 1969. p 176.

7-Breitmaier E. and Voelter W. Carbon-13 NMR Spectroscopy, ed. Weinheim: VCH, 1987. p 381.

8-Billets S., Lietman P.S., Fenselau C. Mass Spectral Analysis of Glucuronides. J. Med. Chem.1973, 16: 30-33.

9-Monti J.P., Gallice P., Crevat A., Murisasco A. Identification by Nuclear Magnetic Resonance and Mass Spectrometry of a Glucuronic Acid Conjugate of o-Hydroxybenzoic Acid in Normal Urine and Uremic Plasma. Clin. Chem. 1985; 31: 1640-1643.

Received: July 11, 1995
Accepted: August 25, 1995