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^{13}C NMR SPECTRA OF SOME SERJANIC ACID DERIVATIVES

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Key Word Index—*Phytolacca octandra*; Phytolaccaceae; methyl serjanate; methyl acetyl serjanate; methyl-3-keto serjanate; methyl acetyl-11-keto serjanate.

Abstract—Full assignments have been made for the ^{13}C NMR signals of methyl serjanate, methyl acetyl serjanate, methyl-3-keto serjanate and methyl acetyl-11-keto serjanate. These data are useful for structural determination without previous chemical degradation of the saponins obtained from members of the Phytolaccaceae.

INTRODUCTION

^{13}C NMR spectroscopy is a powerful tool in elucidating structures of natural triterpene saponins without previous chemical degradation. Several papers have been published in this field [1–5] and in the last few years a number of new structures of triterpene saponins have appeared in the literature [6–9]. Several saponins with interesting biological properties, for instance with anti-inflammatory and molluscicidal activity, have been isolated from the Phytolaccaceae family [10, 11]. In our laboratory we isolated several triterpenic saponins of *Phytolacca octandra* which showed fungostatic activity against *Tricophyton* subsp. [12]. The principal feature of sapogenins of the Phytolaccaceae family, such as phytolaccinic acid, phytolaccagenin and serjanic acid is the presence of two carboxyl groups one of which is on C-17 and the other on C-20. However, in the literature there are no full assignments of the ^{13}C NMR chemical shifts for the carbons on the E ring for these types of compounds. It is well known that some sapogenins suffer important changes in their structures in acidic or basic media. In this paper, full assignments are given for the ^{13}C NMR signals of methyl serjanate and some derivatives. These data will be useful for elucidation of the structure of saponins isolated from the Phytolaccaceae family without previous chemical degradation.

DISCUSSION

For unequivocal determination of each carbon of the molecule, compounds **1**, **2**, **3** and **4** were synthesized and their ^{13}C NMR spectra determined (Table 1). In the acetylated compounds there are differences in the chemical shifts of C-1, C-2, C-4 and C-24. This fact is in agreement with the observed results for oleanolic acid and some other pentacyclic triterpenes. The product of the Jones' oxidation of serjanic acid allowed us to assign C-10, C-23 and C-25 and to corroborate the spectral positions of C-1, C-2 and C-4 [1, 4].

From the revision of literature data, discrepancies in optical rotation [13] of synthetic derivatives of serjanic acid were observed, for which reason identification of asymmetric centres C-8, C-9, and C-14 were made with methyl acetyl-11-keto serjanate (**4**). The chemical shifts of these carbons were in good agreement with the reported values for methyl glycyrrhate. In the same compound the stereochemistry of the D/E ring junction was confirmed as *cis* for an observed eclipsed ε long-range interaction

Table 1. ^{13}C NMR chemical shifts for serjanic acid derivatives.

	Compound			
Carbon	**1**	**2**	**3**	**4**
C-1	38.4	38.1	39.0	38.7
C-2	27.1	23.1	34.0	23.1
C-3	78.7	30.7	216.9	80.4
C-4	38.7	37.6	46.7	38.0
C-5	55.2	55.2	51.4	55.0
C-6	18.3	18.2	19.5	18.8
C-7	32.6	32.6	32.1	32.7
C-8	39.2	39.2	39.2	43.2
C-9	47.5	47.5	47.3	61.6
C-10	36.9	36.8	36.7	37.1
C-11	23.3	23.2	23.3	199.7
C-12	122.9	122.9	122.9	128.0
C-13	142.9	142.9	142.9	167.2
C-14	41.4	41.4	41.5	44.9
C-15	27.6	27.6	27.6	27.6
C-16	23.3	23.3	23.4	23.4
C-17	46.0	46.0	46.0	45.5
C-18	42.5	42.5	42.5	42.7
C-19	42.0	42.0	41.9	40.3
C-20	43.6	43.7	43.6	43.5
C-21	30.3	30.3	30.3	30.3
C-22	33.4	33.4	33.5	32.8
C-23	27.9	27.9	26.6	28.0
C-24	15.6	16.7	21.4	16.6
C-25	15.3	15.4	14.9	16.2
C-26	16.7	16.7	16.6	17.3
C-27	25.9	25.8	25.7	23.1
C-28	176.4	176.4	176.4	176.0
C-29	28.1	28.3	28.3	28.1
C-30	177.3	177.3	177.3	176.6
OMe	51.7	51.6	51.6	51.8
	51.4	51.4	51.4	51.8
COMe	–	21.2	—	21.2

Chemical shifts in ppm from TMS. All signals were corroborated from off-resonance decoupling experiments.

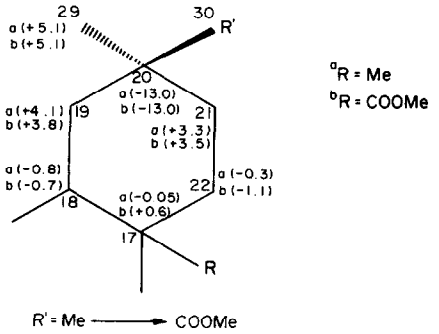
between the hydrogens of the methyl group on C-14, and the hydrogens over C-19 which showed a shielding effect in both carbons similar to that observed in the δ eclipsed interaction [14]. This fact determines the stereochemistry of the D/E ring junction, since this interaction is only possible in a *cis*-junction. This shielding effect is the same as that observed for the transformation of methyl glycyrrhate into 11-deoxo methyl glycyrrhate [5].

For the exact location of the carboxyl group at position C-29 or C-30 of the E ring and the assignments of C-20, C-21 and C-22 a comparative study was carried out of the $\Delta\delta$ values obtained in the conversion of β-amyrin into methyl glycyrrhate against the results obtained for conversion of methyl oleanate into methyl serjanate. The results obtained are similar qualitatively but not quantitatively, as seen in Fig. 1. Using these data, the correct spatial position of the methoxycarbonyl group on C-20 was corroborated as β such as in methyl glycyrrhate.

In the results shown in Fig. 1 it can be seen that the most perturbed carbon is C-20 which is directly linked to the new functional group. The values of $\Delta\delta$ obtained for this carbon are in very good quantitative correlation those obtained for the conversion of β-amyrin into methyl glycyrrhate. However, if the values obtained for this carbon are compared with those obtained for the methyl glycyrrhate into methyl serjanate conversion, a difference of +0.6 ppm is observed. This discrepancy between the two values can be explained by an interaction between two bulky methoxy carbonyl groups. In methyl glycyrrhate this interaction is not present since in this compound C-28 is not oxidized and for this reason the interaction between two methoxy carbonyl groups is not present. This hypothesis was corroborated for the $\Delta\delta$ values obtained for C-17, which is the base for the other methoxy carbonyl group. For this carbon in the conversion of β-amyrin into methyl glycyrrhate, the $\Delta\delta$ value is negligible. However, in methyl serjanate the $\Delta\delta$ has a value of +0.6. It is notable that this value is the same as that obtained for C-20 in the conversion of methyl glycyrrhate into methyl serjanate.

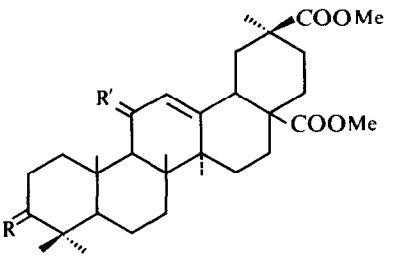
All the other carbons in the E ring were also slightly displaced with respect to the results obtained for methyl glycyrrhate with the exception of C-18 which does not have conformational freedom.

It is interesting to observe the effect of a double bond on C-13 which is thus displaced by 1.5 ppm with regard to the compound without this functional group. Deformation in the E ring causes changes in the spatial position of the hydrogens on C-19, which shows a known steric



[a]R = Me
[b]R = COOMe

Fig. 1.



	R	R'
1	αH βOH	H_2
2	αH βOAc	H_2
3	O	H_2
4	αH βOAc	O

interaction with the hydrogen on C-13. This effect was also observed for methyl acetyl-11-keto serjanate which shows a downfield shift of 2.1 ppm with regard to methyl glycyrrhate. For the above-mentioned reasons, a deformation in the E ring caused by the introduction of a bulky group on C-20 is proposed. This deformation promoted perturbation mainly in the carbons of the E ring.

EXPERIMENTAL

^{13}C NMR spectra were obtained with a Varian XL 100 AFT operated at 25.2 Hz. Chemical shifts are in ppm from int. TMS. The samples were run at concns of *ca* 0.5 mM in $CDCl_3$.

Serjanic acid was obtained from *Phytolacca octandra* berries as reported earlier [15]. Methyl ester **1** and methyl acetyl ester **2** were synthesized as usual (ethereal CH_2N_2 and pyridine–Ac_2O). Jones oxidation of methyl serjanate gave methyl-3-keto serjanate **3** [13]. Allylic oxidation of methyl acetyl serjanate produced the methyl acetyl-11-keto serjanate **4** [16]. The mps of all compounds were identical with those reported in the literature.

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CAMPESTERYL BEHENATE, A CHEMICAL CHARACTER OF THE LIVERWORT GENUS *CALYPOGEIA**

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Key Word Index—*Calypogeia integristipula*; *C. neesiana*; *C. sphagnicola*; Marchantiopsida; liverworts; chemotaxonomy; campesteryl behenate; phytosterol.

Abstract—The discovery of campesteryl behenate in all species of the *Calypogeia* genus so far studied suggests that it is a characteristic feature of this liverwort genus.

INTRODUCTION

As a contribution to the chemotaxonomy of the liverwort [1], we have studied the composition of three species of *Calypogeia*, namely *C. integristipula* Steph., *C. neesiana* (Mass. et Carest.) K. Muell. and *C. sphagnicola* (Mass. et Carest.) Warnst. et Loeske. Our knowledge of the chemistry of this genus originates from Huneck [2,3], who found 1,4-dimethylazulene, 4-methyl-1-methoxycarbonylazulene and 3,7-dimethyl-methoxycarbonylindene in *C. trichomanis* (L.) Corda and unidentified bluish compounds [4] in *C. goebelii* (Schifn.) Steph. In *C. trichomanis*, *C. muelleriana* (Schifn.) K. Muell. and *C. integristipula* Steph., the presence of campesteryl behenate was recorded [5]. In preliminary work, one of us [6] reported the same compound in *C. meylanii* Buch; but this plant is now more correctly known as *C. integristipula*; the content of *n*-alkanes, cerides and their fatty acids in the petrol extract of this plant was published later [7].

RESULTS AND DISCUSSION

All the species now studied yielded as the main and typical component of their petrol extracts, after chromatography on silica gel, the same identical crystalline compound (mp 96–98°; hexane). Spectral data showed the presence of a sterol esterified by an aliphatic acid. After saponification, the presence of docosanoid (behenic) acid, identified as its methyl ester, and of campesterol, was proved. In this sterol, originating from all the species studied, an admixture of *ca* 3% sitosterol was detected, using GC. By another identification using reduction by $LiAlH_4$, and following acetylation [8], docosanyl and campesteryl acetates were obtained.

Consequently, the most significant compound present in all three species is campesteryl behenate. The presence of this sterol ester in all 5 species (of about 8 species registered in Europe) so far analysed suggests it is characteristic for the genus *Calypogeia*.

EXPERIMENTAL

For CC, Si gel (30–60 μm prepared according to ref. [9] and deactivated with 10% of water) was used; prep. HPLC was performed on Separon Si VSK (Laboratorní přístroje Praha): 10 nm in a column 8 × 500 mm, solvent *n*-hexane + 0.5% isopropanol. GC analyses were carried out on a PYE Series 104/64, on 3% SE-30 on GasChrom P at 250° (for sterols) or 210° (acid methyl esters). For TLC, Si gel G Merck was used.

Plant material. C. integristipula was collected in September 1970 near Jestřebice in Central Bohemia, *C. neesiana* and *C. sphagnicola* in August 1976 near Hora sv. Šebestiána in the Ore Mountains. *Campesteryl behenate*—carefully selected liverworts were dried at room temp., ground in a ball-mill, and extrd with

* Part 263 in the series "Terpenes". For Part 262 see Beneš, I., Vaněk, T., Budesinsky, M. and Herout, V. (1981) *Phytochemistry* (in press).