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THE ISOLATION OF METHYL β-ORSELLINATE FROM *STEREOCAULON ALPINUM* AND COMMENTS ON THE ISOLATION OF 4,6-DIHYDROXY-2-METHOXY-3-METHYLACETOPHENONE FROM *STEREOCAULON* SPECIES

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Key Word Index—*Stereocaulon alpinum*; Stereocaulaceae; phenolic carboxylic acids; depsides; atranorin; 4,6-dihydroxy-2-methoxy-3-methylacetophenone; methyl β-orsellinate; ^{13}C NMR.

Abstract—Methyl β-orsellinate has been isolated from chloroform extracts of *Stereocaulon alpinum*. It is shown that methyl β-orsellinate was probably previously misidentified as 4,6-dihydroxy-2-methoxy-3-methylacetophenone during work on *Stereocaulon vesuvianum*. Reports of the isolation of the latter thus appear to be erroneous. The status of methyl β-orsellinate as a naturally occurring metabolite is discussed.

INTRODUCTION

Lichens contain a range of unique aromatic derivatives often linked together as esters known as depsides and depsidones [1]. Their presence may have taxonomic significance. Thus, *Stereocaulon alpinum* Laur. is characterized by the presence of atranorin together with, in one chemical race, lobaric acid, and in the other, stictic acid [2]. Some of these compounds are reported to have antibacterial activity [3]. Thus we have investigated the antimicrobial activity of many species of lichens growing in Iceland. This has involved the successive extraction of dried specimens with solvents of increasing polarity and testing the extracts against certain Gram-negative bacteria and fungi. The results of that study will be published elsewhere.

However, during the course of this work, we have re-investigated the stability of some depsides to the usual extraction procedures. The results of this study form the present communication.

RESULTS AND DISCUSSION

Extraction of a dried specimen of *S. alpinum* with petrol (bp 40–60°) followed by chloroform allowed isolation of atranorin (**1**) together with another material which, on the basis of melting point, mass spectral and ^{1}H NMR data, appeared to be identical to the acetophenone (**2**) already reported as a constituent of *S. vesuvianum* Pers. [4]. Comparative ^{1}H NMR data is shown in Table 1 but unfortunately a sample of this latter material was not available for direct comparison. However, since it has been reported that depsides may undergo alcoholysis by heating with alcohols or alcohol-containing solvents [5] it was considered possible that this material could really be methyl β-orsellinate (**3**), an isomer of the substituted acetophenone. This suggestion has been made previously [6]. The former could be produced by alcoholysis of atranorin.

In the study of Bolognese *et al.* [4], this could occur since the lichen was extracted with ethanol and, in our present investigation, by extracting with chloroform, since this solvent contains a small amount of ethanol as stabilizer.

Differentiation of isomers such as methyl β-orsellinate and the substituted acetophenone is difficult by ^{1}H NMR spectroscopy and depends on the assignment of the methyl signals at δ2.45 and 3.92 (see Table 1). The problem is facilitated, however, by the use of ^{13}C NMR spectroscopy. Bolognese *et al.* did not report ^{13}C NMR data, but the spectral data for aromatic carbons of the compound isolated in the present study are reported in Table 2 together with predicted ^{13}C NMR chemical shift values for both isomers [7]. It is readily seen that the data calculated for C-4 of methyl β-orsellinate more closely resembles those observed for the substance obtained in this present study than those for the acetophenone.

The ^{13}C NMR spectra of the two compounds could be

Table 1. Comparative ^{1}H NMR data of methyl β-orsellinate (**3**) and data reported [4] for 4,6-dihydroxy-2-methoxy-3-methylacetophenone (**2**)

3		**2**	
Chemical shift (δ)	Assignment	Chemical shift (δ)	Assignment [4]
12.04	Ar-OH	11.98	Ar-OH
6.20	Ar-H	6.19	Ar-H
5.23	Ar-OH	5.19	Ar-OH
3.92	$-CO_2Me$	3.90	–OMe
2.45	Ar-Me	2.44	–COMe
2.10	Ar-Me	2.10	Ar-Me

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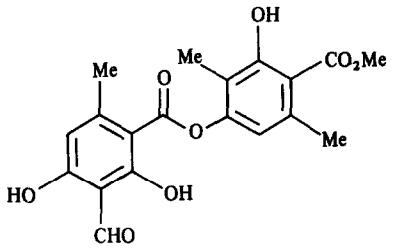

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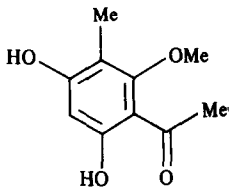

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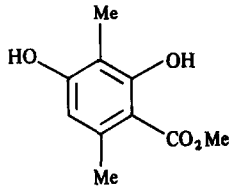

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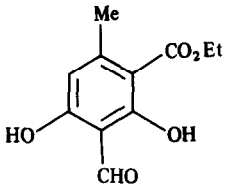

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Table 2. ^{13}C NMR data for aromatic carbons of methyl β-orsellinate (3) and 4,6-dihydroxy-2-methoxy-3-methylacetophenone (2)*

	Chemical shifts (δ, $CDCl_3$)		
C	Calc. for 3	Calc. for 2	Observed†
1	110.9	103.1	105.2
2	158.4	163.5	158.0
3	108.9	103.1	108.5
4	162.7	126.7	163.1
5	108.9	95.3	110.5
6	138.3	155.0	140.2

*Assignments calculated using ref. [7].

†Some of these signals have been assigned differently [8, 9].

distinguished further by the chemical shift of the carbonyl carbons, as ketones give signals considerably further downfield (*ca* δ196) than do esters (*ca* δ167). The observed signal at δ172.6 would thus imply the presence of an ester rather than a ketone. A ^{13}C NMR spectrum of the acetophenone derivative would also reveal a second signal in the δ45–60 region for the methoxyl group at C-2 instead of the observed signal at the higher field of δ24 for the C-6 methyl group.

The observations discussed above seem to confirm that the compound isolated in the present study is methyl β-orsellinate. Furthermore, it is likely that the structure of the compound previously isolated by Bolognese *et al.* from *S. vesuvianum* is wrong and that this is also methyl β-orsellinate (3) rather than the isomeric acetophenone (2) previously reported [4].

The UV absorption data for the two isomers differ somewhat. Bolognese *et al.* [4] reported the absorption maximum at 247 nm (log ε4.2) compared with 267 nm (log ε4.0) found in the present study. (However, the maximum is shifted to 245 nm on the addition of alkali in both cases.) Absorption maximum calculations [10] give 268 nm for methyl β-orsellinate and 288 nm for the acetophenone derivative. The former obviously fits well with the data obtained in the present study. The data of Bolognese *et al.*, although somewhat lower, certainly are more comparable with the data calculated for methyl β-orsellinate than those for the acetophenone.

The next question that arises concerns the status of methyl β-orsellinate since, because it may be produced by hydrolysis of atranorin, it may be that the substance is an artefact. Trials were carried out which consisted of heating atranorin with ethanol and with various grades of chloroform. Ethanolysis occurred to some extent under all the conditions tried, including using the supposedly alcohol-free chloroform prepared by the methods of the *British Pharmacopoeia* and Vogel [11, 12]. TLC examination of the reaction mixture showed the presence of three components: one corresponding to the starting atranorin, one identical to that of the material newly isolated from the plant (shown here to be methyl β-orsellinate), and traces of a third which was presumably due to the other ethanolysis product, ethyl haematommate (4).

The results indicate that despite these precautions some alcoholysis nonetheless always occurs and reports of the isolation of substituted aromatic compounds which could theoretically be produced by ethanolysis of depsides must be treated with caution.

It is thus suggested that a previous report of the isolation of methyl β-orsellinate as a naturally occurring metabolite from *Parmelia tinctorum* [13] could also be erroneous and that the compound was formed from the boiling chloroform employed in the purification process. Thus, rapid extraction and check TLC investigations may not always be a reliable guide to those compounds actually present in the plant (cf. refs. [4, 5]).

EXPERIMENTAL

^{13}C NMR: 100.6 MHz (Brucker WH400; atranorin) or at 50.3 MHz (Nicolet NT200; methyl β-orsellinate); ^{1}H NMR: 400 MHz (Bruker WH400; atranorin) or at 200 MHz (Nicolet NT200; methyl β-orsellinate). TMS (δ0.0) was used as internal standard. MS (VG Micromass 16S): 70 eV; mps are uncorr.

Extraction of S. alpinum. The lichen material was collected from the moraines of Solheima glacier, V. Skaftafellssysla, Iceland in July and August 1980. The material was identified by Professor H. Kristinsson, Institute of Biology, University of Iceland. A herbarium specimen of the lichen has been deposited at the museum at Chelsea College, University of London. The plant was air-dried at ambient temp. to obviate thermally-induced decomposition [14] and thoroughly cleansed of all extraneous material prior to being ground into a fine powder. The powdered lichen thallus (800 g) was extracted successively with the following solvents: petrol (bp 40–60°), $CHCl_3$, Me_2CO, EtOH, MeOH and H_2O. The residue from evapn of the filtered $CHCl_3$ extract was chromatographed through a column of silica

gel G. Elution with hexane followed by $CHCl_3$ and increasing concns of MeOH in $CHCl_3$ allowed the isolation of several compounds.

Atranorin (1) was recrystallized from C_6H_6 yielding colourless needles (290 mg), mp 195–197°; IR $\nu_{max}^{Solid\ film}$ cm^{-1}: 2915, 1655, 1650, 1635, 1287, 1170, 1100; UV λ_{max} nm (log ε): 251 (4.0), 277 (4.0); ^1H NMR ($CDCl_3$): δ 11.24, 10.70, 10.65 (each 1H, *s*, Ar-OH, exchangeable with D_2O), 10.36 (1H, *d*, *J* = 0.7 Hz, CHO), 6.52 (1H, *dd*, *J* = 0.7, 0.4 Hz, Ar-H, H-5'), 6.40 (1H, *dd*, *J* = 0.7, 0.7 Hz, Ar-H, H-5), 3.98 (3H, *s*, $-CO_2Me$), 2.69 (3H, *d*, *J* = 0.7 Hz, Ar-Me, C-6), 2.54 (3H, *dd*, *J* = 0.7, 0.7 Hz, Ar-Me, C-6'), 2.09 (3H, *d*, *J* = 0.4 Hz, Ar-Me, C-3'); ^{13}C NMR ($CDCl_3$): δ 193.8, 172.2, 169.7, 169.1, 167.5, 162.9, 152.4, 152.0, 139.9, 116.8, 116.0, 112.8, 110.2, 108.5, 102.8, 52.3, 25.6, 24.0, 9.4; MS *m/z* (rel. int.): 374 $[M]^+$ (1 5), 196 (75), 179 (14), 165 (19), 164 (100), 150 (17), 136 (51).

Methyl β-orsellinate (3) was recrystallized from Me_2CO yielding pale-green prismatic rods (175 mg), mp 140–141°; IR $\nu_{max}^{Solid\ film}$ cm^{-1}: 3400, 1630, 1500, 1445, 1310, 1115, 800; UV λ_{max} nm (log ε): 267 (4.0), 299 (3.4); ^1H NMR ($CDCl_3$): δ 12.04 (1H, *s*, Ar-OH, C-2, exchangeable with D_2O), 6.20 (1H, *s*, Ar-H), 5.23 (1H, *s*, Ar-OH, C-4, exchangeable with D_2O), 3.92 (3H, *s*, $-CO_2Me$), 2.45 (3H, *s*, Ar-Me, C-6), 2.10 (3H, *s*, Ar-Me, C-3). ^{13}C NMR ($CDCl_3$): δ 172.6, 163.1, 158.0, 140.2, 110.5, 108.5, 105.2, 51.8, 24.1, 7.6; MS *m/z* (rel. int.): 197 $[M+1]^+$ (6), 196 $[M]^+$ (49), 165 (26), 162 (100), 137 (10), 136 (99), 108 (10), 107 (10), 83 (11), 44 (48), 43 (17). Accurate mass measurement: found: 196.0737; $C_{10}H_{12}O_4$ requires: 196.0736.

Alcoholysis of atranorin. To establish how sensitive atranorin is to the presence of EtOH, the following experiments were performed. (a) 50 mg atranorin was refluxed in reagent grade $CHCl_3$ for 3 hr. TLC examination on silica gel G and GF_{254} using toluene as mobile phase revealed that two major hydrolysis products had formed, one of which had TLC properties identical to those of methyl β-orsellinate. (b) Atranorin was refluxed in redistilled $CHCl_3$ with results similar to those described in (a) above. (c) Atranorin was refluxed in 'EtOH-free $CHCl_3$' prepared in accordance with the method of the *British Pharmacopoeia* [11]. This also gave similar results to the previous two experiments. (d) Another method of removing EtOH from $CHCl_3$ was tried [12], which involved washing the $CHCl_3$ three times with conc. H_2SO_4, six times with H_2O and subsequently drying it over Na_2SO_4. Atranorin was refluxed in this purified $CHCl_3$ under N_2 for 3 hr. Results showed that although the amount of EtOH present was minute from IR studies, nonetheless it was sufficient to cause partial alcoholysis of the atranorin, as shown by check TLC as in (a).

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