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REVISION OF STRUCTURE OF RANGIFORMIC ACID

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Key Word Index—*Cladia retipora*; lichen; (+)-rangiformic acid; structural revision; biological activity.

Abstract—On the basis of new NMR evidence, the structure of the lichen-substance, (+)-rangiformic acid, should be revised to (2*S*,3*S*)-(+)-1-methoxycarbonylheptadecane-2,3-dicarboxylic acid. Its biological activities against a range of organisms are recorded.

INTRODUCTION

While screening New Zealand plants and lichens for bioactive constituents we noted antimicrobial activity in ethanolic extracts of *Cladia retipora* [1]. This lichen had been reported to contain atranorin, usnic acid and rangiformic acid [2]; upon fractionation of lichen extracts we obtained the same three compounds. The antimicrobial properties of usnic acid and antranorin are well known [3–5], but little seems to have been reported about rangiformic acid, other than that it showed modest antibacterial activity towards *Staphylococcus aureus* [3, 4]. We report herein a reinvestigation of the structure of rangiformic acid and some further biological activities.

RESULTS AND DISCUSSION

Rangiformic acid was first isolated by Paterno from the lichen, *Cladonia rangiformis* [6]. It was subsequently recognized to be a mono-methyl ester of a heptadecane-1,2,3-tricarboxylic acid, *nor*-rangiformic acid [7, 8], whose stereochemistry was shown to be 2*S*,3*S* by enantiospecific synthesis [9]. A regio-isomeric methyl ester, (+)-*iso*rangiformic acid, was subsequently discovered in another lichen, *Lecanora stenotropa* [10]. As *iso*rangiformic acid yielded a 6-membered cyclic anhydride (as shown by IR ν_{max} 1758 and 1790 cm^{-1}), it was identified as (2*S*,3*S*)-2-methoxycarbonylheptadecane-1,3-dicarboxylic acid (**1**) [10]. Rangiformic acid is therefore either **2** or **3**. It was first deduced to be the 3-methoxycarbonyl compound (**2**) on the basis of its negative ion mass spectrum [11] and this conclusion was subsequently supported by the following argument.

In accordance with expectation, rangiformic acid formed a 5-membered cyclic anhydride (ν_{max} 1774 and 1844 cm^{-1}), which must be **4** or **5**. Selective irradiation of the methoxyl protons was said to result in a carbonyl ^{13}C resonance changing from a complex signal to a doublet, a result interpreted as revealing that the anhydride was **4** and that rangiformic acid was (2*S*,3*S*)-3-methoxycarbonylheptadecane-1,2-dicarboxylic acid (**2**) [12].

This analysis requires the ester carbonyl carbon to have a significant $^2J_{C,H}$ coupling to H-3, with negligible $^3J_{C,H}$ couplings to H-2 and 4. As it is known that $^2J_{C,H}$ and $^3J_{C,H}$ are often comparable in magnitude [13–15], this requirement seemed improbable. In our hands, repetition of Huneck and Steglich's selective proton-carbon decoupling experiment on the anhydride did not simplify the carbonyl carbon to a doublet but instead gave a quartet-like multiplet. This left open the matter of whether rangiformic acid was **2** or **3**.

Unlike the situation with $CHCl_3$-*d* or benzene-d_6 as solvents, in pyridine-d_5 solution at 400 and 300 MH_z, rangiformic acid gave ^1H NMR spectra in which the resonances for H-1, 2, 3, 4 and 5 were resolved. In the corresponding ^{13}C-NMR spectra, the three carbonyl groups were also well separated. By COSY, DEPT and HETCOR spectra, the ^1H and ^{13}C resonances for the C-1 to C-5 terminal unit were assigned (Table 1).

Our first approach to the assignment of the carbonyl resonances used selective INEPT (SELINEPT) [16, 17] measurements, optimised for a $J_{C,H}$ of 5 Hz.

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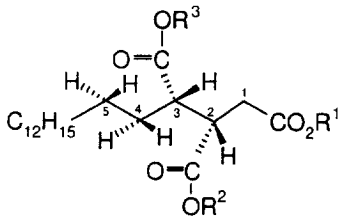
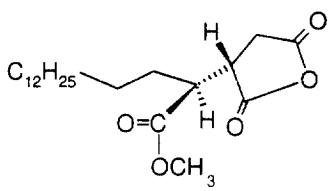


1. $R^1 = R^3 = H$, $R^2 = CH_3$

2. $R^1 = R^2 = H$, $R^3 = CH_3$

3. $R^2 = R^3 = H$, $R^1 = CH_3$



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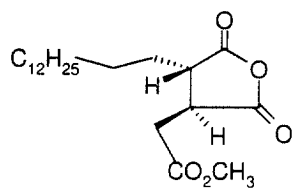

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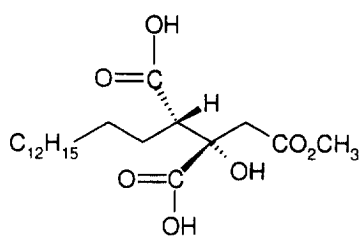

6.

Irradiation of the methoxyl protons resulted in selective enhancement of the resonance at δ_C 173.5, i.e. this was identified as the carbonyl of the ester functionality. Similarly irradiation of H-1A (δ_H 3.33) or H-1B (δ_H 3.08) resulted in the enhancement of both the ester carbonyl and another at δ_C 176.5, whilst irradiation of H-2 (δ_H 4.03) enhanced all three carbonyl resonances and of H-3 (δ_H 3.50) the two carboxylic acid carbonyl resonances at δ_C 176.5 and 177.2. If we exclude esterification of the carboxyl group at C-2 (which corresponds to isorangiformic acid) and accept that $^4J_{C,H} \ll {}^3J_{C,H}, {}^2J_{C,H}$ [13, 14], these results indicate that the esterified carboxyl is at C-1, not C-3.

This conclusion was supported by HMBC spectra [18, 19] which revealed long-range coupling of H-1A and H-1B to C-2 (δ_C 44.4), C-3 (δ_C 47.6), the ester carbonyl (δ_C 173.5) and another at δ_C 176.5, H-2 to C-1 (δ_C 33.6), C-3, C-4 (δ_C 29.3) and the carbonyls at δ_C 173.5, 176.5 and 177.2, H-3 to C-1, C-2, C-4 and δ_C 176.5 and 177.2, as well as the other correlations shown in Table 1. Thus, it is concluded that rangiformic acid has the structure **3**, not **2**.

Some years ago, at a time when rangiformic acid was recognized to be a monomethyl ester of norrangiformic acid but the site of methylation was unknown, it was suggested that it might be **3**, formed from another lichen product, caperatic acid (**6**) [20], by dehydration and reduction [21]. Our demonstration that rangiformic acid is **3** revives this biosynthetic proposal.

Rangiformic acid (**3**) showed marginal activity

Table 1. ^{1}H and ^{13}C NMR assignments for (+)-rangiformic acid (**3**)*

H	δ_H	HMBC correlations (to δ_C)	C	δ_C
1A	3.33 (*dd*, 10.2, 16.7)	44.4, 47.6, 173.5, 176.5	1	33.6
1B	3.08 (*dd*, 4, 16.7)	44.4, 47.6, 173.5, 176.5		
2	4.03 (*ddd*, 4, 4, 10.2)	29.3, 33.6, 47.6, 173.5, 176.5, 177.2	2	44.4
3	3.50 (*ddd*, 4, 4, 6.7)	29.3, 33.6, 44.4, 176.5, 177.2	3	47.6
4A	2.15 (*m*)		4	29.3
4B	1.84 (*m*)			
5A	1.69 (*m*)		5	28.3
6–14	1.2–1.4 (*m*)		6–14	30–30.5
15	ca 1.25 (*n.o*)		15	32.5
16	ca 1.25 (*n.o*)		16	23.3
17	0.87 (*dist.t*, ca 7)	23.3, 32.5	17	14.6
OCH_3	3.63 (*s*)	173.5	OCH_3	51.9
			1-CO_2	173.5
CO_2H	12.2 (2H, *brs*)		2-CO_2	176.5
			3-CO_2	177.2

* In pyridine-d_5 ("100 atom%"), using traces of residual pyridine for internal reference (δ_H 7.22 (H-2) and δC 123.5 (C-2)).

against *Bacillus subtilis* but not against *Candida albicans*, *Cladosporium resinae*, *Escherichia coli*, *Pseudomonas aeruginosa* or *Trichophyton mentagrophytes* (at 60 μg per disk). We ascribe the antimicrobial activity of a crude extract of *C. retipora* [1] to the presence of usnic acid. Rangiformic acid (**3**) was inactive against P388 leukemia cells ($IC_{50} > 25$ $\mu g\ ml^{-1}$) but showed marginal cytotoxicity against monkey kidney (BSC-1) cells (at 60 μg per disk).

EXPERIMENTAL

Rangiformic acid was isolated from a collection of *C. retipora* (Labill.) Nyl from the Maungatua tops, near Dunedin, in March 1996 (Plant Extracts Research Unit voucher specimen 960330-01). It had mp 105.5–107.5° (uncorr.) after recrystallization from EtOAc, $[\alpha]_D$ +22.7° (*c* 0.13, EtOH). Lit. mp 102–104° [12], 104–105° [21], $[\alpha]_D$ +18.2 (EtOH) [22], +14.2° (EtOH) [12]. The anhydride, prep according to ref. [12] and recrystallised from hexane had mp 51–53° (uncorr.); lit. mp 52–54° [12]. ^{1}H and ^{13}C-NMR spectra (Table 1) were recorded with Bruker AM-400 and AMX-300 spectrometers. Bioassays were performed as described in refs [1, 23].

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