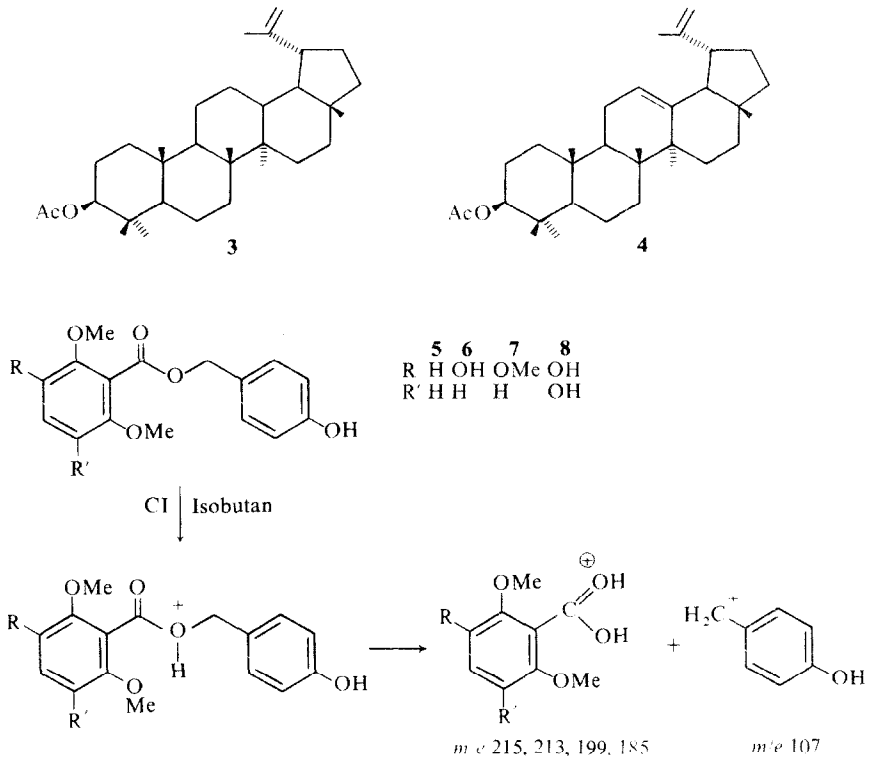

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PROPIONATE PRECURSORS IN THE BIOSYNTHESIS OF DAUNOMYCIN AND ADRIAMYCIN: A ^{13}C NUCLEAR MAGNETIC RESONANCE STUDY

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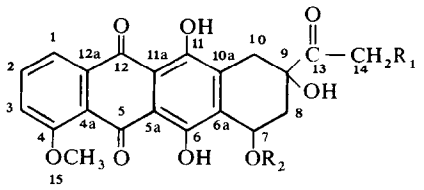
Key Word Index—*Streptomyces peucetius* var. *caesius*; daunomycin; adriamycin; sodium propionate[1-^{13}C]; biosynthesis; ^{13}C-NMR.

Abstract—A ^{13}C-NMR study of the biosynthesis of daunomycin and adriamycin from propionate[1-^{13}C] has been carried out in cultures of *Streptomyces peucetius* var. *caesius*. Results give direct support for the postulate that a propionate 'starter' is involved in the biosynthesis of both metabolites.

Daunomycin (**1**) [1] and adriamycin (**2**) [2] are both anthracycline antibiotics. The polyketide origin of both microbial metabolites has been demonstrated using ^{13}C-NMR in conjunction with [1-^{13}C], [2-^{13}C]- and doubly labelled ^{13}C-sodium acetate incorporation studies into **1** by *Streptomyces peucetius* (ATCC No. 21354) [3]. The labelling pattern was shown to be consistent with a biosynthetic pathway involving a propionate 'starter' and nine successive malonate condensations with the loss of the terminal carboxyl. As part of a related study, we wish to present here the results of propionate incorporation experiments which demonstrate unequivocally the incorporation of sodium propionate [1-^{13}C] into **1** and **2** by cultures of *Streptomyces peucetius* var. *caesius* (IMRU No. 3920).

Carbon-13 enriched **1** and **2** were prepared biosynthetically as described and were analysed by ^{13}C-NMR as their aglycones, daunomycinone (**3**) and adriamycin-



Compound	R_1	R_2
1	H	1'-α-(3-amino-2,3,6-trideoxy-L-lyxosyl)
2	OH	1'-α-(3-amino-2,3,6-trideoxy-L-lyxosyl)
3	H	H
4	OH	H

Table 1. Carbon chemical shifts, Δ (ppm) and labelling pattern found for the aliphatic sidechains of 3 and 4

Carbon No.	Daunomycinone (3) unlabelled	labelled	Adriamycinone (4) unlabelled	labelled
7	60.5		60.4	
8	35.6		36.2	
9		76.2		76.2
10	31.9		32.5	
13	211.8		213.6	
14	24.4		64.1	

Δ run in DMSO-d_6, results are reported relative to TMS using the relationship δ(TMS) = δ(DMSO-d_6) − 39.5 ppm.

one (4), respectively. The ^{13}C-NMR chemical shift assignments for the aliphatic sidechains of both aglycones are summarized in Table 1 and are based upon literature values [4].

A comparison of the intensities of the appropriate signals in spectra of the labelled and unlabelled compounds revealed that, in both cases, the quaternary aliphatic ring carbon (C-9, 76.2 ppm) was enriched *ca* 10-fold. This result constitutes direct support for the postulate that a propionate (C-15, C-14 and C-9) 'starter' is directly involved in the biosynthesis of both daunomycin and adriamycin in this organism. A similar result has been observed for the related metabolite ξ-pyrromycinone using ^{14}C-tracer studies [5].

EXPERIMENTAL

Culture. The source of *Streptomyces peucetius* var. *caesius* (IMRU No. 3920) and conditions used for its maintenance have been described [2].

Isotopic incorporation studies. Submerged production cultures of *S. peucetius* var. *caesius* were grown at 28° in 300 ml flasks containing 40 ml medium and stirred on a shaker rotating at 220 rpm with a 7 cm radius [2]. 48 hr after inoculation, 40 mg sodium propionate [1-^{13}C] (90 atoms % ^{13}C, Merck) was added to each flask. After a further 72 hr incubation the whole culture was exhaustively extracted as previously described [2]. Mild acid hydrolysis of crude 1 and 2 with 0.1 N HCl at 100° for 20 min gave a mixture of dark red coloured aglycones, 3 and 4. Each metabolite was separated and purified by repeated PLC on Si gel developed with $CHCl_3$–Me_2CO (4:1). PFT-^{13}C-NMR spectra were recorded on a Varian XL100 (25.2 MHz) spectrometer using DMSO-d_6 as solvent.

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NEUE TREMETON-DERIVATE AUS *DORINICUM MACROPHYLLUM*

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Key Word Index—*Doronicum macrophyllum*; Senecioneae; Compositae; new tremetone derivatives; benzfurans.

Bisher sind aus Vertretern der Gattung *Doronicum* (Tribus Senecioneae) neben dem weitverbreiteten Pentainen (**1**) [1] aus einer Art Thymol- und Tremeton-Derivate [2] isoliert worden.

Auch die Wurzeln von *Doronicum macrophyllum* enthalten neben **1–4** die Thymolderivate **9–11** und die Tremeton-Derivate **17** und **18**. Daneben isoliert man drei weitere derartige Verbindungen, denen nach den spektroskopischen Daten die Konstitutionen **15**, **16** und **21** zukommen (s. Tabelle 1).

Die oberirdischen Teile, aus denen bereits das Pyrrolizidin-Alkaloid Doronin isoliert wurde [3], ergeben neben **1**, **2–8**, **12** und **16–18** zwei weitere Tremeton-Derivate, denen die Konstitutionen **19** und **20** zukommen (s. Tabelle 1). Die Stellung der phenolischen OH-Gruppe ergibt sich aus der Lage und der Kopplung der aromatischen Protonen-Signale.

Die vorliegenden Ergebnisse geben keine neuen Anhaltspunkte über eine mögliche Umgruppierung dieser Gattung in eine andere Tribus. Ähnliche Tremeton-