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STRUCTURE DETERMINATION OF A 4-PYRONE FROM THE LIVERWORT *PLAGIOCHILA BIFARIA* (SW.) LINDENB. (PLAGIOCHILACEAE)

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SUMMARY

The centenary of the presentation in 1907 of the "polyketide" hypothesis by Collie, along with his use of a 4-pyrone as an example, is marked by reporting the discovery and isolation of 2-(3,4-dimethoxyphenethyl)-6-methyl-4-pyrone. This compound dominates the phytochemical profile of the deuteriochloroform extract of a Venezuelan specimen of *Plagiochila bifaria* and the structure was determined using spectroscopic techniques, especially 2D NMR. This natural product is novel because it contains what appears to be the first example of a monocyclic 4-pyrone that has a polyketide origin. The hypothetical pathway for cyclization of a triketocarboxylic acid to a 4-pyrone was known to be applicable to model systems but no examples of natural products from this route were known.

This compound is the same as one of a series of compounds observed previously in an extract of a Brazilian specimen of *P. bifaria*; the working structures that were proposed earlier require revision. The mass spectral characteristics are the same as those reported (parent and base peak) in 1987 for the major component observed in the GC–MS profile of an extract of a specimen of *P. bifaria* from Peru.

The roles played by Birch and Robinson in the renaissance of the polyketide hypothesis almost fifty years after its initial launch are considered. Based on evidence from their publications, they worked independently of each other. It appears Robinson always had knowledge of Collie's hypothesis when developing ideas about structural relations of natural products whereas Birch initially was unaware of both Collie's and Robinson's ideas on the subject.

KEYWORDS: Natural product chemistry, NMR spectroscopy, Hepaticae, 4-pyranone

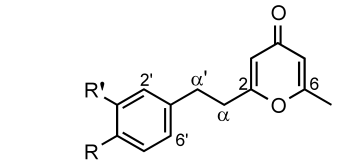
INTRODUCTION

Molecular, morphological and phytochemical evidence (Heinrichs *et al.*, 2004) has supported a broad species concept of *Plagiochila bifaria* (Sw.) Lindenb., a liverwort widespread in the Neotropics and known also from Macaronesia and Atlantic Europe. Phytochemical profiles of $CDCl_3$ (deuteriochloroform) extracts were determined for a range of specimens. Most of them contained several 9,10-dihydrophenanthrenes; some contained, in addition, a few methyl orsellinates, and one specimen, from Brazil, contained a series of compounds, not isolated, for which working structures based on 9-phenylnon-6-en-2-one were proposed. One of these compounds has now been discovered to

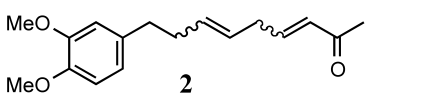
dominate the phytochemical profile of a specimen of *P. bifaria* collected in Venezuela during the XV IAB World Conference in 2004. This compound has been isolated and elucidation of its structure as a 4-pyrone derivative (**1a**) is reported here (chemical structures are identified by bold numerals in Fig. 1). This discovery of a natural 4-pyrone is discussed in the context of polyketide biosynthetic pathways and enlisted to draw attention to the centenary in 2007 of the presentation by Collie (1907a) of what is now recognised as the polyketide hypothesis.

MATERIALS AND METHODS

Plant material was collected from a fallen branch in the La Carbonera – San Eusebio Forest, Mérida State, Venezuela, on 17 January 2004 and dried in the air at ambient temperature. The packet (*Rycroft 040117-1405*) contained tufts of two distinct *Plagiochila* species. Material identified as *P. bifaria* is distinguished as *Rycroft 040117-1405a*.



	R	R'
1a	OMe	OMe
1b	OMe	H
1c	H	H



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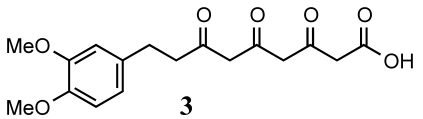

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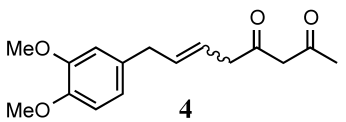

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Fig. 1. Chemical structures.

A $CDCl_3$ extract was prepared on 19 April 2006. Individual shoots were selected and examined with a hand lens to check that all belonged to the same taxon and were clean. The shoots (48 mg) together with a few pieces of broken glass were ground to a powder in a glass vial using a glass rod and extracted with $CDCl_3$ (r.t., *ca* 0.5 h) to give a filtered solution (*ca* 0.7 mL) that was used directly for ^{1}H NMR and GC–MS profiling (Rycroft, 1996).

Conditions for GC–MS profiling were similar to those used previously (Rycroft & Cole, 2001). HREIMS was performed at 70eV. NMR experiments were performed at 9.4 T. NOE (nuclear Overhauser effect) experiments were performed using pulsed field gradient selective excitation (*cf.* Stott *et al.*, 1997).

Compound **1a** was isolated by elution of the extract through a short plug of silica gel supported in a Pasteur pipette, that yielded several fractions (each *ca* 0.7 mL) that were screened directly using ^{1}H NMR spectroscopy. GC–MS showed that the first fraction collected on elution with $CDCl_3$ contained the more volatile components. The next three fractions, also eluted with $CDCl_3$, were essentially clear. The following fraction, eluted with CD_3OD, contained compound **1a**.

GC–MS of $CDCl_3$ extract

All except four peaks are <5% of the TIC (total ion current): $[M]^+$, base peak, TIC %, identity: 204, 91, 7%, unidentified; 204, 121, 5%, bicyclogermacrene; 274, 151, 53%, compound **1a**; 290, 124, 9%, compound **11e** in Heinrichs *et al.* (2004).

2-(3,4-Dimethoxyphenethyl)-6-methyl-4-pyrone (1a)

^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): see Table 1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (1H,

d, J = 8.1 Hz, H-5'), 6.72 (1H, dd, J = 8.1, 2.0 Hz, H-6'), 6.69 (1H, d, J = 2.0 Hz, H-2'), 6.07 (2H, m, H-3, H-5), 3.869, 3.866 (each 3H, s, 3-OMe, 4-OMe), 2.90, 2.78 (each 2H, m, H_2-α, H_2-α'), 2.25 (3H, d, J = 0.8 Hz, 6-Me); EIMS *m/z* 274 $[M]^+$ (51), 259 (5), 243 (2), 181 (12), 152 (24), 151 (100), 124 (14), 107 (16); HREIMS *m/z* 274.1204 $[M]^+$ (calcd for $C_{16}H_{18}O_4$: 274.1205).

RESULTS

Certain parts of the ^{1}H NMR spectrum of the $CDCl_3$ extract of the Venezuelan specimen of *P. bifaria* contained readily interpretable signals. The aromatic region was dominated by the three-proton system of a 1,3,4-trisubstituted benzene ring. Integration relative to the residual $CHCl_3$ proton signal of the $CDCl_3$ solvent showed that the amount of the compound responsible for these signals that had been extracted from the plant was 48 moles per tonne of dry material. In addition, amongst other signals, there were corresponding singlets for two methoxyl groups, two multiplets around δ_H 2.8 and 2.9 ppm corresponding to an isolated CH_2–CH_2 system (confirmed later by 2D ^{1}H–^{1}H correlation spectroscopy), and a methyl resonance appearing as a narrow doublet at δ_H 2.2 (J = 0.8 Hz). The GC–MS profile of this extract (see MATERIALS AND METHODS) was dominated (53% of the TIC) by a peak that had a parent ion $[M]^+$ with *m/z* = 274 and a base peak with *m/z* = 151. GC–MS showed that the fraction of the extract eluted with CD_3OD from the silica gel plug contained mainly the compound with molecular mass 274 Da (designated as compound **1a**). The ^{1}H NMR spectrum of **1a** in this fraction revealed that, in addition to the signals mentioned above, there was a pair of signals around δ_H 6.1 ppm with relative intensities corresponding to one proton each. This fraction was evaporated to dryness and redissolved in $CDCl_3$. Integration of the ^{1}H NMR spectrum of this solution relative to the residual $CHCl_3$ signal showed that the quantity of **1a** present was *ca* 0.5 mg. The pair of signals around 6.1 ppm that were well separated in the ^{1}H NMR spectrum of the CD_3OD solution were barely resolved in the $CDCl_3$ spectrum, so most of the NMR experiments were performed with a CD_3OD solution prepared following evaporation of the $CDCl_3$ solution. On completion of the NMR experiments, the CD_3OD solution was used for GC–MS and HREIMS measurements. Afterwards, the material recovered by evaporation of the CD_3OD was redissolved in $CDCl_3$ and gave a ^{1}H NMR spectrum that was unchanged, with respect to the signals in common, from that of the original $CDCl_3$ extract.

GC–MS of the CD_3OD solution of **1a** showed the presence of a few minor impurities but ion-monitoring showed that the ion with *m/z* = 274 arose only from **1a**. Accurate mass measurement of this ion then showed that the molecular formula of **1a** is $C_{16}H_{18}O_4$.

The ^{1}H and ^{13}C NMR data of **1a** in CD_3OD are in Table 1 (the ^{1}H data from the $CDCl_3$ solution of **1a** are in the MATERIALS AND METHODS). It was impractical to obtain the ^{13}C NMR data by direct observation of the resonance of the ^{13}C magnetisation because only *ca* 0.5 mg of **1a** was available. Instead, indirect methods were used that transfer polarisation from protons and take advantage of their greater magnetisation; these methods, however, are dependent on the presence of nuclear spin-spin coupling between ^{13}C and at least one proton. Sensitivity was still problematical and in a ^{13}C spectrum obtained using DEPT (distortionless enhancement by polarisation transfer) with a 135° read pulse, the only signals observed from **1a** were of five methine carbons in the aromatic region. One-bond ^{13}C–^{1}H correlations for these signals were

Table 1. *NMR spectroscopic data (9.4 T, CD_3OD) for 2-(3,4-dimethoxyphenethyl)-6-methyl-4-pyrone (**1a**). HMBC cross-peaks are from the carbon of the row to the proton(s) stated.*

position	δ_C	type	δ_H	mult. (*J* in Hz)	HMBC
2	171.7	qC			3, α, α′
3	113.9	CH	6.08	d (2.3)	α
4	not obs.				
5	114.0	CH	6.14	dq (2.3, 0.7)	6-Me
6	169.4	qC			5, 6-Me
1'	134.2	qC			5', α, α′
2'	113.6	CH	6.80	d (2.0)	6', α′
3'	150.6	qC			5', 3-OMe
4'	149.2	qC			2', 4-OMe
5'	113.3	CH	6.85	d (8.1)	
6'	121.9	CH	6.73	dd (8.1, 2.0)	2', α′
α	36.5	CH_2	2.88	m	α′
α′	33.8	CH_2	2.93	m	α
6-Me	19.7	CH_3	2.30	q (0.7)	
3-OMe	56.7	CH_3	3.80	s	
4-OMe	56.7	CH_3	3.79	s	

observed in a 2D HSQC (heteronuclear single quantum correlation) spectrum along with those for one methyl, two methylene and two methoxyl groups. Longer range two- and three-bond $^{13}C\cdots^{1}H$ correlations observed in a 2D HMBC (heteronuclear multiple bond correlation) spectrum enabled the ^{13}C chemical shifts of five quaternary carbons to be determined. Interpretation of the observed NMR data up to this point had accounted for a portion of the molecular formula $C_{16}H_{18}O_4$ amounting to $C_{15}H_{18}O_2$.

Structural elucidation continued as follows. The three-proton system characteristic of a 1,3,4-trisubstituted benzene ring enabled the assignments of H-2', H-5' and H-6'. Observation of positive NOEs at only H-2' and H-5' on saturation of both methoxyl proton signals located the methoxyl groups at C-3' and C-4'. The positions were distinguished on the basis of three-bond HMBCs from C-3' to one of the methoxyl proton signals and to H-5' and from C-4' to the other methoxyl proton signal and to H-2'. C-1' was assigned from a three-bond HMBC to H-5'. Attachment of the 2,3-dimethoxyphenyl group to a CH_2–CH_2 moiety followed from HMBCs of C-1' to H_2-α and H_2-α′. Three-bond HMBCs from C-2' and C-6' to H_2-α′ assigned the latter. C-2 was identified by virtue of another HMBC involving H_2-α′ that must be through three bonds. Similarly, HMBC of H_2-α to a carbon that had not yet been assigned must also be through three bonds and identified C-3; in addition C-2 correlates with H-3. Chemical shifts show that C-2 and C-3 are olefinic and the deshielded nature of C-2 indicates attachment to oxygen. The small coupling (2.3 Hz) between H-3 and H-5 (both olefinic methine protons) indicates a four-bond separation. There is coupling also between H-5 and the 6-methyl protons. This, together with NOE enhancement of H-5 upon selective excitation of the 6-methyl protons and HMBCs between C-6 and both H-5 and the 6-methyl protons, suggested a double bond between C-5 and C-6. The deshielded chemical shift of C-6, very similar to that of C-5, also indicates attachment to oxygen. Bearing in mind the unassigned elements (one carbon and two oxygen atoms) and that some moiety

is required to separate C-3 and C-5, the structure of compound **1a** may be completed by inserting an oxygen atom between C-2 and C-6 and a carbonyl group between C-3 and C-5. The result is a 2,6-disubstituted 4-pyrone structure and it then becomes clear why H-3 and H-5, C-3 and C-5, and C-2 and C-6 have such similar pairs of chemical shifts. Comparison with the literature showed that the NMR data are very similar to those of 2,6-dimethyl-4-pyrone (Pouchert & Behnke, 1993) and 2-methyl-6-phenethyl-4-pyrone (**1c**) (only ^{1}H data available: Yamamoto *et al.*, 1986). The chemical shift of C-4 ought to be around 180 ppm but the absence of any HMBCs of C-4 involving H-3 and H-5 does not affect the arguments leading to structure **1a**, which is 2-(3,4-dimethoxyphenethyl)-6-methyl-4-pyrone.

DISCUSSION

The structure of compound **1a** is new. No natural products with similar structures are known but two synthetic compounds are closely related. One is 2-*p*-methoxyphenethyl-6-methyl-4-pyrone (**1b**), synthesised during a study of the components of kava root (Borsche & Blount, 1932). The other is 2-methyl-6-phenethyl-4-pyrone (**1c**), synthesised by Birch, Cameron & Rickards (1960) in order to convert it to 8-phenyloctane-2,4,6-trione and on to dihydro-pinosylvin, a component of pine heartwoods.

Compound **1a** is the same as one of the components of an extract of a Brazilian specimen (*Costa & Gradstein 3711*) of *P. bifaria* studied in previous work (Heinrichs *et al.*, 2004; Rycroft, 2005); there the compound was not isolated and structure **2** was presented as a working hypothesis. The main thesis of that work however was that minor changes in biosynthetic pathways may result in grossly different outcomes and that such phytochemical variation does not compromise the broad species concept of *P. bifaria*. Determination of the structure of this compound as **1a** rather than **2** does not affect the conclusions of that work. Indeed, structure **1a** is a more satisfactory outcome of the speculative biosynthetic pathway discussed previously (Rycroft, 2005) in that the oxygen atoms occur more where they would be expected for a polyketide pathway.

As noted previously (Heinrichs *et al.*, 2004; Rycroft, 2005), the mass spectral characteristics of **1a** (referred to previously by the incorrect structure **2**) are the same as those (parent and base peak) reported twenty years ago for the major component observed in the GC–MS profile of an extract of a specimen (*Inoue 34048*) of *P. bifaria* from Peru (Asakawa & Inoue, 1987). It therefore seems likely that the chemotype of *P. bifaria* that produces compounds such as **1a** occurs in Peru as well as Brazil and Venezuela, and may be widespread in the Neotropics.

Establishment of structure **1a** necessitates reconsideration of the structures of the other components of the extract from the Brazilian specimen *Costa & Gradstein 3711*. The compounds designated **11b**, **11e**, **11f** by Heinrichs *et al.* (2004) may have 4-pyrone structures analogous to that of **1a** (**11d** in Heinrichs *et al.*, 2004). The other two compounds, **11a** and **11c**, apparently have two more hydrogen atoms than their putative 4-pyrone counterparts (**11b** and **11d**). The biogenesis of compound **1a** is considered below, but structure **1a** can formally be regarded as the product of intramolecular condensation and decarboxylation of the linear β-triketocarboxylic acid 9-(3,4-dimethoxyphenyl)-3,5,7-trioxononanoic acid (**3**) and plausible structures for the putative dihydro-compounds may formally be derived from the corresponding linear β-triketocarboxylic acids by reduction, dehydration and

decarboxylation, leading amongst other possibilities to such as structure **4** for **11c**. This structure is speculative and more evidence is required, especially with respect to the positions of the double bond and the carbonyl groups.

The 4-pyrone structure of the natural product **1a** is of considerable interest. The probable polyketide biosynthesis of such a structure proceeds in general (Fig. 2) by condensation of an alkanoyl-CoA starter unit with three units of malonyl-CoA to form a linear tetraketide chain; to form **1a**, the starter unit might be dihydro-*p*-coumaroyl-CoA or a close derivative. Thirty years ago, Harris & Harris (1977) reviewed the four theoretical dehydrative cyclizations of such a linear tetraketide (see Scheme in Fig. 2) and said that, while natural products from three of the routes were known, "the natural occurrence of

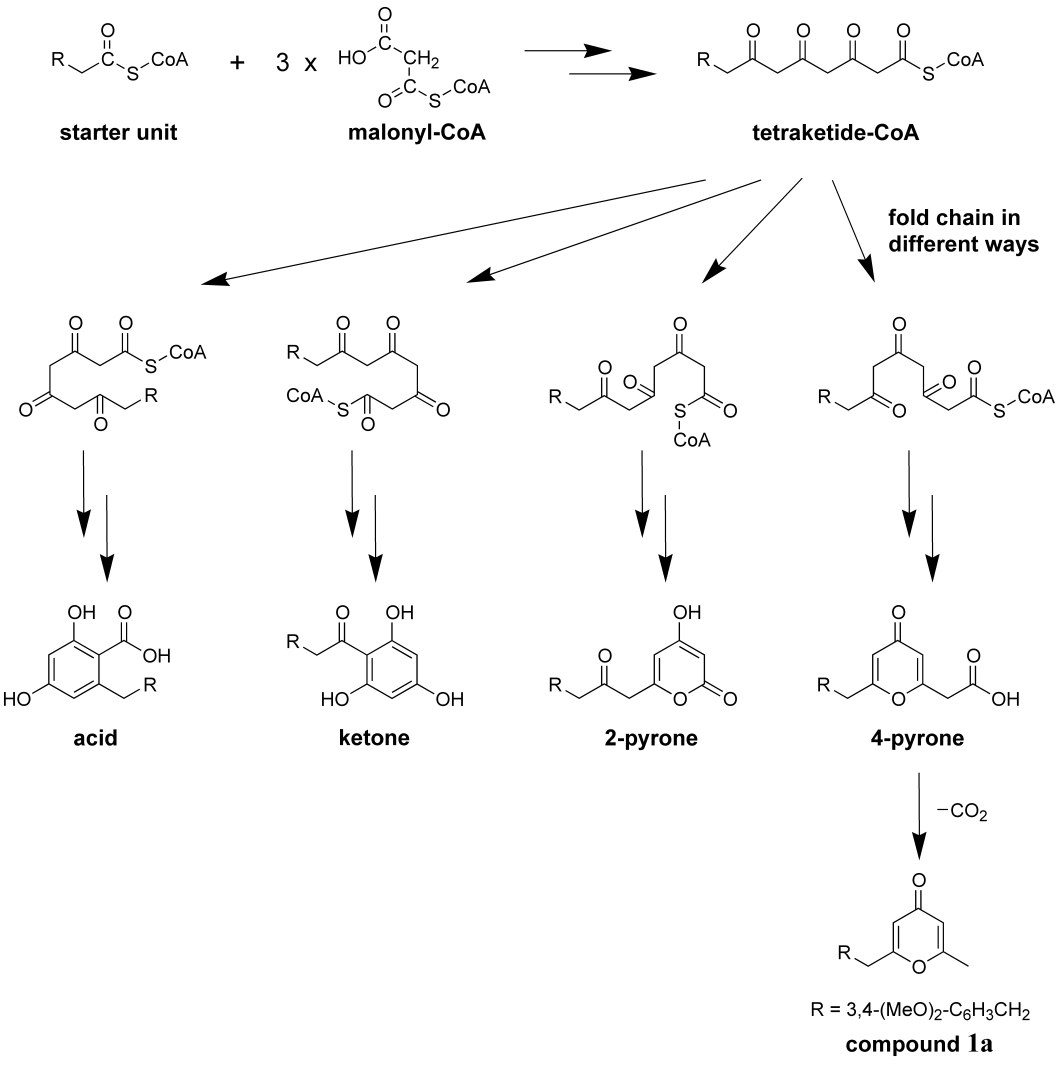

Fig. 2. Schematic biosynthetic and cyclization pathways to tetraketide-derived secondary metabolites.

polyketide-derived 4-pyrones is in doubt." This view was restated *ca* twenty years ago (Harris & Harris, 1986), by noting that 4-pyrones as naturally occurring polyketide products had "not been observed in monocyclic systems", and appears to hold true until now. One possibility that has to be considered in the case of **1a** is that one of the regular biosynthetic pathways may have become blocked at some point and that cyclization of an intermediate linear polyketide related to **3** may occur by spontaneous chemical reaction rather than by enzyme-mediated processes; whether the final decarboxylation step that is required would occur spontaneously is more questionable. Formation of such a derailment product would be somewhat analogous to the isolation by Gatenbeck *et al.* (1969) of 2,6-dimethyl-4-pyrone upon denaturation of an enzyme system that would otherwise have produced a benzenoid product. In that example however the conditions were rather severe: heating in 6M HCl for 30 min! In the present work the plant extract was produced under mild conditions and it seems reasonable to regard **1a** as a natural product, whether or not all steps of its biosynthesis are under enzymatic control.

Polyketide biosynthesis was the subject of a recent millennium review (Staunton & Weissman, 2001), which includes some information on the early development of the subject. In addition it is of interest to note that the year 2007 is the centenary of the coining of the term "polyketide" by the multi-talented Collie (1907a) in a paper read to the Chemical Society in London on 24 October 1907. His ideas that reactions of polyketides in the laboratory may be related to reactions occurring in plants had been developing over several years (Collie, 1893). One of the compounds used to illustrate his paper was 2,6-dimethyl-4-pyrone, that could be synthesised from tetra-acetic acid by decarboxylation (to diacetylacetone) and dehydration in acidic solution. In the discussion following delivery of this paper there was criticism from Forster (one of the Honorary Secretaries of the Chemical Society) that the scope of Collie's "entirely novel expression" was not defined precisely enough. Maybe this is why the term "polyketide" does not appear in the full version of the paper published in the Transactions, being replaced by "multiple keten" (Collie, 1907b). The term was only reinstated when Birch (1962) delivered the Simonsen Lecture on 4 May 1961, also to the Chemical Society. Collie's ideas were apparently ahead of his time and had been largely ignored for over forty years. Robinson included a brief mention of similar ideas in a lecture delivered to the Royal Society of Arts in London on 21 April 1948 (the year after receiving the Nobel Prize for Chemistry in recognition of his work on plant products of biological importance, especially alkaloids), but only in the context of having said that there was no strong evidence in favour of either this view or an alternative route from C_6 sugars. There is no mention of Collie in the publication of that lecture (Robinson, 1948), but Robinson succeeded Collie as Professor at University College London in 1928 and, in answer to the question "was [Robinson] aware of Collie's earlier speculations?" posed by Staunton and Weissman (2001), it seems clear that he was always aware of Collie's ideas because he stated in the publication (Robinson, 1955) arising from the First Weizmann Memorial Lectures (delivered by Robinson in December 1953), that "the polyacetic acid hypothesis has been in the minds of chemists since J. N. Collie propounded it in 1907." Meanwhile, Birch & Donovan (1953) in Australia had re-stated the polyketide hypothesis independently and conducted labelling

experiments to provide experimental support. Collie (1907b) and Robinson (1948) are both cited in the 1953 paper, but Birch (1957) emphasised in a review that "Of Collie's work we were unaware until it was pointed out to us by Dr. P. Maitland (Cambridge)." To understand the context of this remark it should be noted that Birch worked for his doctorate with Robinson at Oxford but had moved to Cambridge in 1949 (Rickards, 1992). In his Simonsen Lecture, Birch (1962) evidently still felt the need to explain that his own work was initiated in 1951 (he returned to Australia in 1952). He again referred to Collie's hypothesis of which "we were initially unaware" and noted that "An apparently independent treatment of the subject by Robinson, of which we were unaware, was not published until 1955, by which time we had already provided considerable experimental support for our hypothesis." Birch made no comment on Robinson's remark, present in the 1955 publication he was citing, about the common currency of Collie's work.

Moving on from the apparent tensions associated with the reintroduction of the polyketide hypothesis, it is a pleasure to be able to celebrate the centenary of Collie's coining of the terms "ketide" and "polyketide" by reporting the discovery of a 4-pyrone natural product from the liverwort *Plagiochila bifaria*.

Norman Collie was one of the top rock climbers and mountaineers of his day. He spent the last three years of his life at Sligachan (Isle of Skye, Scotland), where he died in 1942. There are plans (http://skyesculpture.com) to erect a bronze sculpture there of both him and the local John MacKenzie, overlooking the Cuillin range, where there are peaks, Sgurr Thormaid and Sgurr Mhic Coinnich respectively, named after them.

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