

***N*-Acetylcolchinol Methyl Ether – a Natural Product:**

Suhailamine – a Phantom Natural Product

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ABSTRACT: The reported characterisation data for the allocolchicinoid alkaloid suhailamine, isolated from *Colchicum decaisnei* Boiss. and known to have an erroneous structure, has been re-analysed. This analysis has led to the current proposal that suhailamine has the same structure as *N*-acetylcolchinol methyl ether (NCME), an assertion which is supported by comparison with previously reported data for NCME. Suhailamine is therefore a phantom natural product, whilst NCME represents a naturally occurring allocolchicinoid rather than a purely synthetic entity.

The first isolation of colchicine (**1**), the main alkaloid of the poisonous plant meadow saffron (*Colchicum autumnale* L.), is usually attributed to Pelletier and Caventou, who reported the results of their studies in 1820.¹ It was Geiger, however, who was first to use the name colchicine after isolation of the pure compound in 1833.² The structure of colchicine (**1**) then baffled chemists for many years and it was only in 1945 (125 years after the report of Pelletier and Caventou appeared) that Dewar proposed the correct structure;³ this was then established conclusively by King *et al.*, who reported their X-ray crystallographic studies in 1952.⁴ Today, colchicine (**1**) is a well-known natural product: it has been the target of several synthetic endeavours⁵ and is recognized both as a treatment for gout and for its antimitotic activity.⁶ As a drug it has truly ancient origins, being referenced in several classical Greek texts, for example in the writings of Pliny the Elder (who died in AD 79 during the cataclysmic eruption of Mount Vesuvius that famously destroyed the cities of Pompeii and Herculaneum),⁷ although in these historic texts it is more often referenced as a poison rather than as a remedy. The use of colchicine (**1**) in the treatment of gout is usually attributed to von Störck in 1763,⁷ and colchicine-based medications have been continuously employed as a remedy for this condition since the early 19th century. It was observed to suppress malignant tumour growth in 1932 by Dominici⁸ and its mode of action (mitotic poisoning by inhibiting tubulin polymerisation) was determined soon after.^{9,10} Animal studies showed that the high toxicity of the compound rendered it unsuitable for cancer chemotherapy,¹¹ although its mode of action has rendered it an important tool in biochemistry and in the development of anticancer drugs.^{12,13} Whilst colchicine (**1**) is too toxic to be used as a treatment for cancer, related alkaloids and colchicine derivatives have been investigated. One of the first examples was the use of the alkaloid demecolcine (**2**) as a treatment for myeloid leukemia,¹² and further interest blossomed in the 1990s when it was discovered that allocolchicinoids—synthetic derivatives of colchicine (**1**) in which the tropolone ring is replaced by a benzene ring, and which sometimes referred to as dibenzocycloheptylamines—also bind to tubulin,¹³ but with reduced toxicity.^{14,15} Examples of allocolchicinoids include allocolchicine (**3**) itself, *N*-acetylcolchicinol (**4**), and *N*-acetylcolchicinol methyl ether (**5**)—commonly abbreviated to NCME and also referred to as *N*-acetyl-*O*-methylcolchicinol or NSC 51046 (Figure 1). Such was the interest in these compounds that ZD6126 (**6**), a prodrug of *N*-acetylcolchicinol (**4**), was advanced to phase II clinical trials for potential use in the treatment of metastatic renal cell carcinoma and metastatic colorectal cancer by AstraZeneca. However, these trials were subsequently halted when it became apparent that ZD6126 (**6**) was, similar to colchicine (**1**), too cardiotoxic at the required doses.¹⁶

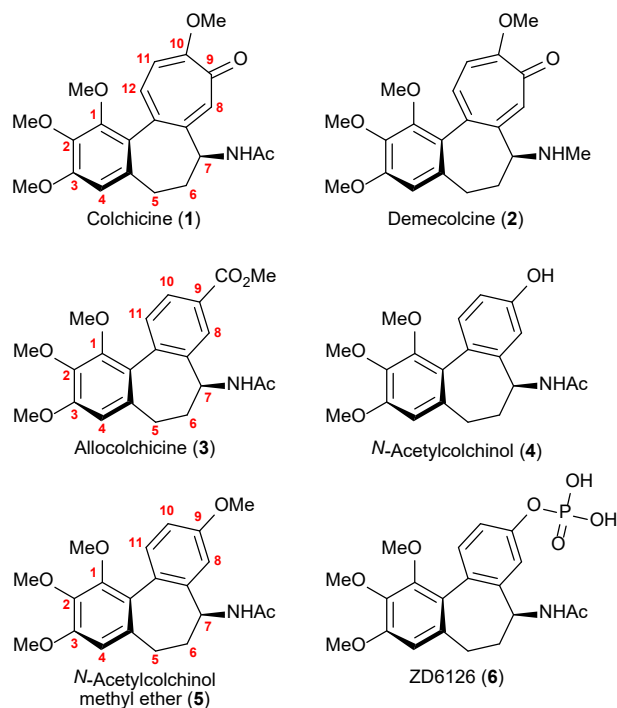


Figure 1. Structures of colchicine (1), demecolcine (2) and representative allocolchicinoids 3–6, depicted as the (a*R*,7*S*)-atropisomeric forms.

The allocolchicinoids (e.g., 3–6) can be produced via laboratory total synthesis, or alternatively from rearrangement or degradation of colchicine (1).¹⁷ As they have been suggested as catabolic metabolites of colchicine (1), however, they also have potential to occur as natural products. Indeed, an interesting contribution to the area was made by Abu Zarga *et al.* in 1991, who investigated the alkaloid content of *Colchicum decaisnei* Boiss. and succeeded in isolating three natural products.¹⁸ The structures of these alkaloids were assigned on the basis of mass spectrometric analyses and a range of different NMR spectroscopic analyses; all three were determined to be allocolchicinoids and named jerusalemine, salimine, and suhailamine (Figure 2). The structure of jerusalemine (7) was subsequently validated upon synthesis by Banwell *et al.*,¹⁹ although in the same study the structure of salimine was found to be erroneous. Banwell *et al.* re-analysed the data reported by Abu Zarga *et al.* for this alkaloid, proposed the structure of salimine (8), and confirmed this structure by its synthesis. In the same study,¹⁹ Banwell *et al.* commented that the structure of suhailamine is also clearly erroneous as it purportedly shares the same structure as allocolchicine (3), although the two compounds have significantly different spectroscopic data. Although this discrepancy has been noted by others,²⁰ hitherto the true identity of suhailamine remains a mystery.

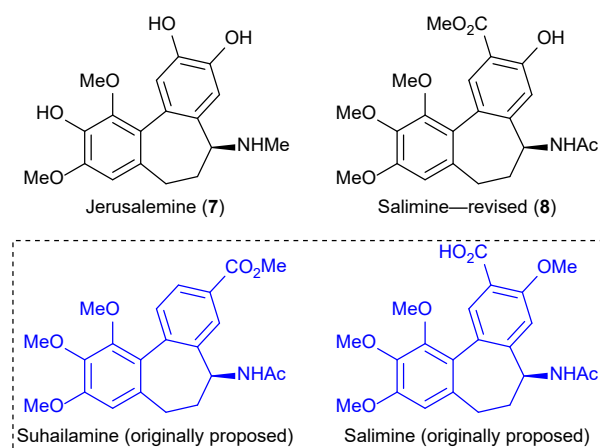


Figure 2. Naturally occurring allocolchicinoids: structures of jerusalemine (7), revised structure of salimine (8), and originally proposed (erroneous) structures of suhailamine and salimine.

Given our recent involvement in the structural reassignment of the Hancock alkaloid galipeine,²¹ we became interested in the case of suhailamine and sought to resolve the question surrounding its structure. Through a re-analysis of the characterisation data reported by Abu Zarga *et al.*,¹⁸ we herein propose that suhailamine shares its structure with that of NCME (5). This hypothesis is supported by comparison of the spectroscopic data reported by Abu Zarga *et al.*¹⁸ for their natural allocolchicinoid with data for NCME (5) reported by Brecht *et al.*²² and Takubo *et al.*²³ for their synthetic samples of NCME (5). Hence, suhailamine is established as a phantom natural product whilst NCME (5) is simultaneously established as a naturally occurring allocolchicinoid.

RESULTS AND DISCUSSION

Initially, a review of the data for suhailamine reported by Abu Zarga *et al.*¹⁸ was undertaken. The molecular formula C₂₂H₂₅NO₆ (RMM = 399) is proposed from analysis of the EIMS data, with the peak of highest mass appearing at *m/z* 399. This peak has only 10% relative intensity, however, whilst the peak of next highest mass, appearing at *m/z* 371, has 80% relative intensity. In order for the latter to represent a daughter ion, the proposed structure of suhailamine would be required to eject a mass of 28 upon fragmentation, which would suggest a loss of CO. This seemed unlikely, given the proposed structure, and instead it was reasoned that it was more likely that the peak of highest mass appearing at *m/z* 399 arose from the presence of an impurity such as colchicine (1)—which has molecular formula C₂₂H₂₅NO₆ and RMM = 399. Such an assertion is not unreasonable as colchicine (1) is known to be found in large amounts in members of the *Colchicum* genus (indeed it can be considered as a chemotaxonomical marker).⁶ It was then proposed that the peak appearing at *m/z* 371 corresponded to the molecular ion of suhailamine; with this assumption, suhailamine would have the molecular formula C₂₁H₂₅NO₅ (RMM = 371) and thus lack a CO unit from the originally proposed formula. Indeed, none of the characterisation data reported by Abu Zarga *et al.*¹⁸

supports the presence of the C=O unit of the ester functionality: peaks in the infrared spectrum at 1610 cm^{-1} and 1590 cm^{-1} are too low, and the reported ^{13}C NMR spectroscopic data do not contain a resonance characteristic of the carbon atom of an ester carbonyl.²⁴

Meanwhile, re-analysis of the ^1H NMR spectroscopic data reported by Abu Zarga *et al.*¹⁸ appeared to be consistent with the basic framework of an allocolchicinoid (dibenzocycloheptylamine) framework, with the observed ^1H – ^1H vicinal couplings and NOE data being consistent with the proposed substitution pattern. On this basis it was hypothesised that rather than a methoxycarbonyl group being present at C-9, a methoxy group is instead present. Analysis of the observed ^1H and ^{13}C NMR chemical shifts of 10-C/H and 11-C/H were supportive of this assignment and this is most clearly illustrated by comparison with the chemical shift data (in CDCl_3) of a range of model compounds **9–16**,²⁵ having either a methoxycarbonyl or methoxy group, built up from methyl benzoate (**9**) and anisole (**13**), respectively. The ^1H and ^{13}C NMR spectroscopic data for suhailamine have a much better match with the model compounds **13–16** having a methoxy group at C-9 (allocolchicinoid numbering) than with the model compounds **9–12** having a methoxycarbonyl group at C-9 (allocolchicinoid numbering): the ^1H and ^{13}C NMR chemical shifts of 10-C/H and 11-C/H clearly show suhailamine (for which 10-C/H appears at δ_{H} 6.84 and δ_{C} 110.8, with 11-C/H appearing at δ_{H} 7.42 and δ_{C} 131.4) is within or closer to the shift range of the model compounds **13–16** having a methoxy group at C-9 (for which 10-C/H appears at δ_{H} 6.77–6.99 and δ_{C} 114.1–114.3, with 11-C/H appearing at δ_{H} 7.33–7.54 and δ_{C} 128.3–129.5) than the model compounds **9–12** having a methoxycarbonyl group at C-9 (for which 10-C/H appears at δ_{H} 7.95–8.11 and δ_{C} 129.3–130.3, with 11-C/H appearing at δ_{H} 7.43–7.67 and δ_{C} 127.2–128.4). If one compares these data with those of allocolchicine (**3**),²⁵ the shifts of 10-C/H (δ_{H} 7.98, δ_{C} 130.2) and 11-C/H (δ_{H} 7.57, δ_{C} 127.5) fall within the shift range of esters **9–12** and not within the shift range of ethers **13–16** (Figure 3).

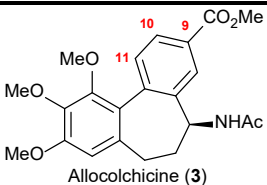
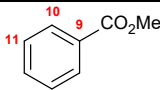
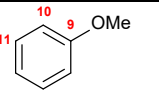
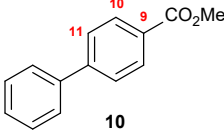
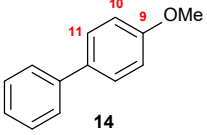
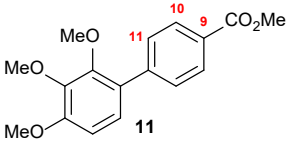
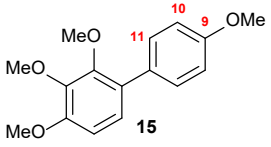
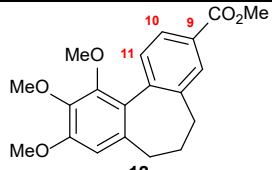
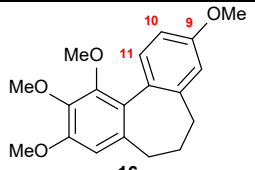
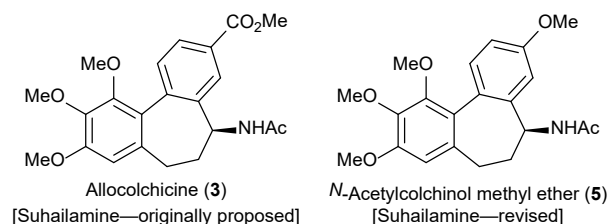
9-CO ₂ Me	¹ H NMR shifts /ppm		¹³ C NMR shifts /ppm		9-OMe	¹ H NMR shifts /ppm		¹³ C NMR shifts /ppm	
	10-H	11-H	C-10	C-11		10-H	11-H	C-10	C-11
 Alcolchicine (3)	7.98	7.57	130.2	127.5	Suhailamine	6.84	7.42	110.8	131.4
 9	8.04	7.43	129.7	128.4	 13	6.90	7.30	114.1	129.5
 10	8.11	7.67	130.2	127.2	 14	6.99	7.54	114.3	128.3
 11	8.07	7.59	129.3	129.0	 15	6.96	7.44	^a	^a
 12	7.95	7.55	130.3	126.9	 16	6.77	7.33	^a	^a

Figure 3. Selected ¹H and ¹³C NMR spectroscopic data (recorded in CDCl₃) for compounds alcolchicine (3), suhailamine, and 9–16. ^aNo ¹³C NMR spectroscopic data have been reported for 15 and 16.

Taken together, these observations provide strong support for the proposal that suhailamine is likely to be the 9-methoxy counterpart of alcolchicine (3), a.k.a. NCME (5). The latter is known to exist as a mixture of rotamers/atropisomers in CDCl₃ (presumably due to restricted rotation around both the amide N–CO bond and the biaryl axis), but essentially as a single entity in either methanol-*d*₄ or DMSO-*d*₆. For ease of analysis, therefore, NMR spectroscopic data for NCME (5) have almost always been recorded and reported using DMSO-*d*₆ as the solvent.²⁶ Only two reports, by Brecht *et al.*²² and Takubo *et al.*,²³ have reported NMR spectroscopic data for their synthetic samples of NCME (5) in CDCl₃ and, until now, no comparison between these data for NCME (5) and those reported for suhailamine has been made.

Brecht *et al.* reported that NCME (5) exists in a 5:2 ratio of rotamers/atropisomers in CDCl₃,²² whilst Takubo *et al.* determined a 13:5:2 ratio of rotamers/atropisomers in the same solvent;²³ it is notable that the 13:5 ratio of the two most populated species in the latter case corresponds well with the 5:2 ratio determined in the former case. As may be expected, therefore, data reported for the major rotameric/atropisomeric form in both studies are in agreement.^{22,23} Meanwhile, comparison of the ¹H NMR spectroscopic data for suhailamine reported by Abu Zarga *et al.*¹⁸ against those of the major rotameric/atropisomeric form reported by both Brecht *et al.*²² and Takubo *et al.*²³ displays $|\Delta\delta_{\text{H}}| \leq 0.07$,²⁷ with the sole exception of the shift of the NH proton (for which $\Delta\delta_{\text{H}} = -0.14$ ²⁷ and $\Delta\delta_{\text{H}} = -0.20$,²⁷ respectively). This particular shift is, however,

likely dependent on the degree of hydrogen bonding, which may be affected by residual water and/or the concentration of the sample (Figure 4).

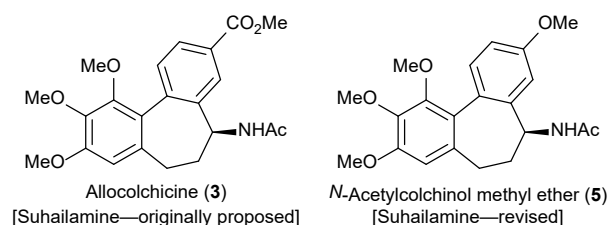


¹H NMR (CDCl₃)

Suhailamine	5 (Ref 20)	5 (Ref 21)
1.77 (1H, m)	1.75 (1H, m)	1.81 (1H, m)
2.05 (3H, s)	2.00 (3H, s)	2.04 (3H, s)
2.35 (1H, m)	2.28 (1H, m)	2.43 (3H, m)*
2.41 (1H, m)	2.34 (1H, m)	2.43 (3H, m)*
2.45 (1H, m)	2.41 (1H, m)	2.43 (3H, m)*
3.52 (3H, s)	3.49 (3H, s)	3.51 (3H, s)
3.85 (3H, s)	3.81 (3H, s)	3.84 (3H, s)
3.89 (3H, s)	3.86 (3H, s)	3.89 (3H, s)
3.92 (3H, s)	3.90 (3H, s)	3.92 (3H, s)
4.78 (1H, m)	4.77 (1H, m)	4.80 (1H, m)
5.71 (1H, br d)	5.91 (1H, d)	5.85 (1H, d)
6.55 (1H, s)	6.53 (1H, s)	6.56 (1H, s)
6.80 (1H, d)	6.80 (1H, d)	6.89 (2H, m)*
6.84 (1H, dd)	6.82 (1H, dd)	6.89 (2H, m)*
7.42 (1H, d)	7.39 (1H, d)	7.42 (1H, d)

Figure 4. Comparison of ¹H NMR spectroscopic data for suhailamine and **5** in CDCl₃. Midpoints of all multiplets are reported for ease of comparison. Reference frequencies are unknown in all three cases, and therefore data are uncorrected. * Overlapping signals.

Similarly, the partial ¹³C NMR spectroscopic data for suhailamine reported by Abu Zarga *et al.*¹⁸ also match the corresponding resonances reported for NCME (**5**) by Brecht *et al.*²² and Takubo *et al.*,²³ with all but one of the carbons displaying $|\Delta\delta_C| \leq 0.3$,²⁷ well within the limits of experimental error, especially given that the reference frequencies used in these cases cannot be confirmed. The one exception relates to the peak reported for suhailamine at $\delta_C = 29.7$, which displays $\Delta\delta_C = -0.9$ ²⁷ and $\Delta\delta_C = -0.7$ ²⁷ when compared to the data reported by Brecht *et al.*²² and Takubo *et al.*,²³ respectively. Interestingly, however, colchicine (**1**) has a shift for C-5 in this region,²⁸ and the presence of colchicine (**1**) in the sample of suhailamine isolated by Abu Zarga *et al.*¹⁸ has already been speculated based on the accompanying mass spectrometric data. Although this evidence is purely anecdotal, it may account for the observed discrepancies between these data sets (Figure 5).



¹³ C NMR (CDCl ₃)		
Suhailamine	5 (Ref 20)	5 (Ref 21)
29.3	29.3 (+0.1)	29.3 (0.0)
29.7	30.6 (- 0.9)	30.4 (- 0.7)
39.8	39.5 (+0.3)	39.5 (+0.3)
49.4	49.3 (+0.1)	49.3 (+0.1)
55.3	55.2 (+0.1)	55.3 (0.0)
56.3	56.1 (+0.2)	56.1 (+0.2)
60.9	60.9 (0.0)	61.0 (- 0.1)
61.2	61.2 (0.0)	61.2 (0.0)
108.0	107.7 (+0.3)	107.9 (+0.1)
109.2	109.2 (0.0)	109.2 (0.0)
110.8	110.6 (+0.2)	110.5 (+0.3)
131.4	131.2 (+0.2)	131.3 (+0.1)

Figure 5. Comparison of (partial) ¹³C NMR spectroscopic data for suhailamine and **5** in CDCl₃. Values of Δδ_C are given in parentheses. Reference frequencies are unknown in all three cases, and therefore data are uncorrected.

The specific rotation for suhailamine reported by Abu Zarga *et al.* was [α]_D –22 (*c* 0.13, MeOH),^{18,29} and this can be compared to values for synthetic samples of the (7*S*)-isomer of NCME (**5**) that have been reported in the same solvent: [α]_D²⁰ –88.6 (*c* 0.67, MeOH),³⁰ [α]₅₉₀¹⁷ –94 (*c* 0.67, MeOH),³¹ [α]₅₉₀¹⁶ –92 (*c* 0.67, MeOH).^{31,32} Although these values are somewhat different in magnitude when compared to the value for suhailamine, the identical signs of all these specific rotation values suggest that the configuration of NCME (**5**)—suhailamine—as a natural product should be assigned as (7*S*), making it homochiral with respect to the naturally-occurring enantiomer of colchicine (**1**); this would be consistent with these natural products being biosynthetically related.

In conclusion, therefore, all of the data contained herein support the assertion that the structure of the allocolchicinoid alkaloid suhailamine is congruent with that of *N*-acetylcolchinol methyl ether (NCME). Whilst this renders suhailamine a phantom natural product, NCME has been established as a naturally occurring allocolchicinoid in *Colchicum decaisnei* Boiss.

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Notes

The authors declare no competing financial interest.

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(24) In fact, the ^{13}C NMR spectroscopic data reported for suhailamine do not encompass any of the signals arising from fully-substituted carbon centres; it was supposed that this was due to them being too weak to be distinguished from the baseline when considering the small amount of material isolated (7 mg) and the sensitivity of the equipment that was likely available in 1991.

(25) Data for **9**, **10**, **13**, and **14** were acquired in this study. Other data are taken from Ref 17c, Ref 17d, and the following additional sources: (a) Itoh, Y.; Brossi, A.; Hamel, E.; Lin, C. M. *Helv. Chim. Acta* **1988**, 71, 1199–1209. (b) Medrano, F. J.; Andreu, J. M.; Gorbunoff, M. J.; Timasheff, S. N. *Biochemistry* **1989**, 28, 5589–5599. (c) Boyé, O.; Itoh, Y.; Brossi, A. *Helv. Chim. Acta* **1989**, 72, 1690–1696.

(26) For example, see Ref 17b and Ref 20.

(27) $\Delta\delta_X = \delta_X(\text{suhailamine}) - \delta_X(\text{synthetic } \mathbf{5})$, where X = H or C.

(28) For example, in Ref 17b this shift is reported at $\delta_C = 29.8$ (CDCl_3).

(29) The temperature at which this value was recorded was not reported.

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(32) In the first two cases, the samples were derived from colchicine (**1**).

TOC Graphic:

