Fingerprinting of plant extracts using NMR spectroscopy: application to small samples of liverworts

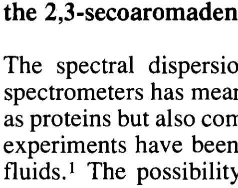
David S. Rycroft

Department of Chemistry, University of Glasgow, Glasgow, UK G12 8QQ

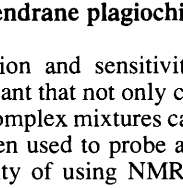
^1H and ^{13}C NMR spectra of $CDCl_3$ extracts of quarter-gram samples of liverworts show that *Adelanthus decipiens* contains 1,2-dimethoxy-3,4-methylenedioxynaphthalene and 1,4-dimethoxy-2,3-methylenedioxynaphthalene, and that *Plagiochila atlantica* contains the 2,3-secoaromadendrane plagiochiline C.

The spectral dispersion and sensitivity of high field NMR spectrometers has meant that not only complex molecules such as proteins but also complex mixtures can be studied, and NMR experiments have been used to probe all manner of biological fluids.[1] The possibility of using NMR spectroscopy to study herbarium material was raised by Phillipson[2] in 1982, but the idea has never been pursued and has made no impact on the development of phytochemistry and chemotaxonomy. The idea is of course applicable to small samples other than actual herbarium specimens, and this communication presents some results concerning liverworts (Hepaticae), which are often available from the field in gram quantities only. Liverworts produce oil bodies which contain terpenoid and/or aromatic compounds, and a wide range of unusual terpenoid skeletal types has been found.[3] Evidence for the biological activity of many of these compounds is nicely provided by the fact that liverwort specimens in herbaria are not prone to insect or fungal attack.[4]

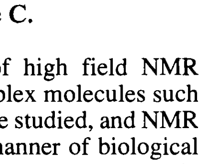
Phillipson extracted herbarium material in the traditional manner but significant benefits accrue if the extraction is performed directly with the NMR solvent. As an example of the results that can be obtained in this new application of NMRspectroscopy, Fig. 1 shows a section of the ^1H NMR spectrum of the filtered solution obtained by triturating 246 mg of *Adelanthus decipiens* (Hook.) Mitt.† with *ca.* 1 ml of $CDCl_3$. This would normally be described as the spectrum of a crude extract but in this case the spectrum is anything but crude. Indeed the aromatic signals are remarkable in their clarity and they can be simulated readily as an 80:20 mixture of the ABMX and AA′XX′ spin systems and chemical shifts shown in **1** and **2** respectively. Methoxy and methylenedioxy signals are also present and the ^1H NMR data correspond to those reported for 1,2-dimethoxy-3,4-methylenedioxynaphthalene (wettstein A, **3**) and 1,4-dimethoxy-2,3-methylenedioxynaphthalene (wettstein B, **4**).[5] In this example the solution was sufficiently concentrated for ^{13}C DEPT experiments to be performed. The ^{13}C as well as the ^1H NMR data show remarkable agreement with the literature,[5] a fact which is attributed to the solute concentrations being low so that intermolecular interactions in the mixture are unimportant. The concentrations can be measured conveniently by comparison of ^1H signals with the residual $^{12}CHCl_3$ or $^{13}CHCl_3$ signals. When the deuterium content is 99.8 atom% the concentration represented by a single ^{13}C satellite is 0.14 mM, and concentrations down to this level are readily measured.‡ In the present case the concentration of **3** was 12 mM. Minor components have not yet been identified.



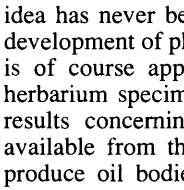
1



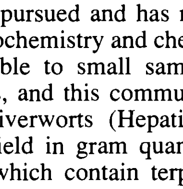
2



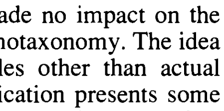
3



4



5



6

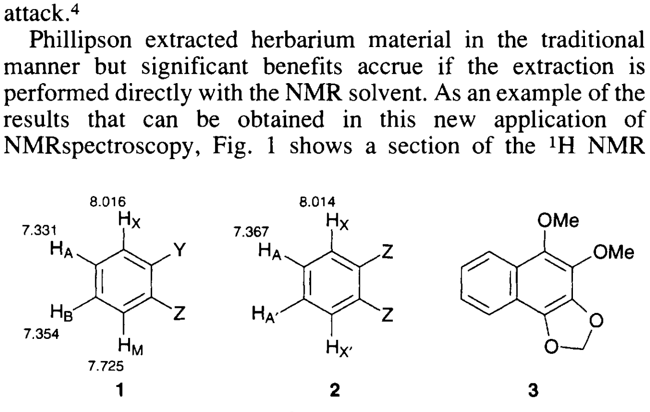


Fig. 1 360 MHz ^1H NMR spectrum of the $CDCl_3$ extract of *Adelanthus decipiens*. The scale shows δ values relative to internal Me_4Si.

The finding of naphthalene derivatives as natural products is unusual and the occurrence of compounds **3** and **4** in *Adelanthus decipiens* is of taxonomic interest. The genus *Adelanthus* belongs to the small family Adelanthaceae;[6] the only other genus belonging to this family is *Wettsteinia*, and the only other report of compounds **3** and **4** is in *Wettsteinia schusterana* from New Zealand.[5] Previous work[7] on *Adelanthus decipiens* (from Colombia) using GC–MS did not result in identification of the major components; as one of these was reported to have the same molecular weight as **3**, the claim that it is a eudesmanolide should be re-examined.

A second example of this new application of NMR spectroscopy is provided by *Plagiochila atlantica* F. Rose. This very rare liverwort§ is most liable to be mistaken[8] for *P. spinulosa*, which has been found[9] to contain derivatives of dihydrophenanthrene and bibenzyl. The ^1H NMR spectrum of the $CDCl_3$ extract of a quarter-gram sample of the liverwort prepared as above showed immediately that the lipophilic compounds of *P. atlantica* are completely different from those of *P. spinulosa*. Aromatic signals were essentially absent. Instead the predominant signals corresponded to those of plagiochiline C,[10] the 2,3-secoaromadendrane derivative **5** (also known as ovalifoliene)[11] that has been isolated from several *Plagiochila* species. The concentration of **5** in the $CDCl_3$ solution was 3 mM and spectral connectivities were proved in a phase sensitive 2D double quantum filtered COSY experiment, which also located overlapping signals.

The processes involved in extraction and isolation of natural products are difficult to control and, for example, volatile components are liable to be lost. The technique described above enables a representative fingerprint of the lipophilic compounds to be obtained very directly from a small sample of plant material. This fingerprint can be very informative if circumstances are favourable and provides a primary point of reference when monitoring the processing of a larger amount of plant material.

The solution from the NMR work can be used directly for GC–MS studies. The new NMR method has some advantages in comparison with GC–MS in that it is not compromised by adsorption and volatility problems, and signals from the whole extract can be seen. The great advantage of GC–MS is that it can usually provide the molecular weight for a compound, and in cases where a GC–MS peak can be related to signals in the NMR spectrum this can be extremely useful, especially if the compound is new. This approach has enabled the new herbertanolide **6** to be identified[12] in the extremely rare liverwort *Herbertus borealis*.

The author would like to thank David G. Long, Royal Botanic Garden Edinburgh, for identifying *Adelanthus decipiens* and for providing a reference specimen of *Plagiochila atlantica*.

Footnotes

† *Adelanthus decipiens*: collection number Rycroft GL1, Glen Loin, Arrochar, 29th October 1994.

‡ Calibration of the $CDCl_3$ showed that the declared residual proton content was correct to within 10%.

§ *Plagiochila atlantica*: collection number Rycroft 96067, Ariundle Wood, Strontian, 26th May 1996.

References

1 J. K. Nicholson, P. J. D. Foxall, E. Holmes, G. H. Neild and J. C. Lindon, in *Magnetic Resonance in Food Science*, ed. P. S. Belton, I. Delgadillo, A. M. Gil and G. A. Webb, Special Publication **157**, RSC, Cambridge, 1995, p. 177.

2 J. D. Phillipson, *Phytochemistry*, 1982, **21**, 2441.

3 Y. Asakawa, in *Progress in the Chemistry of Organic Natural Products*, ed. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich and C. Tamm, Springer, Vienna, 1995, vol. 65, p. 1.

4 The Herbarium Handbook, ed. D. Bridson and L. Forman, Royal Botanic Gardens, Kew, revised edition 1992, p. 236.

5 Y. Asakawa, Y. Tada and T. Hashimoto, *Phytochemistry*, 1994, **35**, 1555.

6 A. J. E. Smith, *The Liverworts of Britain and Ireland*, Cambridge University Press, Cambridge, 1990.

7 S. R. Gradstein, R. Matsuda and Y. Asakawa, *J. Hattori Bot. Lab.*, 1981, **50**, 231.

8 E. W. Jones and F. Rose, *J. Bryology*, 1975, **8**, 417.

9 D. S. Rycroft, in *Bryophytes: Their Chemistry and Chemical Taxonomy*, ed. H. D. Zinsmeister and R. Mues, Oxford University Press, Oxford, 1990, p. 109.

10 Y. Asakawa, M. Toyota, T. Takemoto and C. Suire, *Phytochemistry*, 1979, **18**, 1355.

11 A. Matsuo, K. Atsumi, M. Nakayama and S. Hayashi, *J. Chem. Soc., Chem. Commun.*, 1979, 1010; A. Matsuo, K. Atsumi and M. Nakayama, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2816.

12 D. S. Rycroft and W. J. Cole, unpublished work.

Received, 17th June 1996; Com. 6/04215E