Phytochemistry, 1979, Vol. 18, pp. 1735–1736. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1001-1735 $02.00/0

^{13}C NMR SPECTROSCOPY OF ω-CYCLOALKYL FATTY ACIDS

M. DE ROSA, C. DI PINTO, A. GAMBACORTA and B. NICOLAUS
Laboratorio per la Chimica di Molecole di Interesse Biologico CNR, Arco Felice, Napoli, Italy

(*Received* 26 *February* 1979)

Key Word Index—*Bacillus acidocaldarius*; ω-cycloalkyl fatty acids; ^{13}C NMR spectroscopy.

ω-Cycloalkyl fatty acids are the most abundant components of the lipids in the acidothermophilic bacterium *Bacillus acidocaldarius* [1, 2]. They also occur in trace amounts in deposits of ruminants and in rumen flora [3]. From the exogenous precursors RCO_2H and RCH_2COOH (R_2 = cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl), *Bacillus acidocaldarius* and *Bacillus subtilis* also synthesize a corresponding range of C_{16} to C_{21} semisynthetic ω-cycloalkyl fatty acids [4, 5].

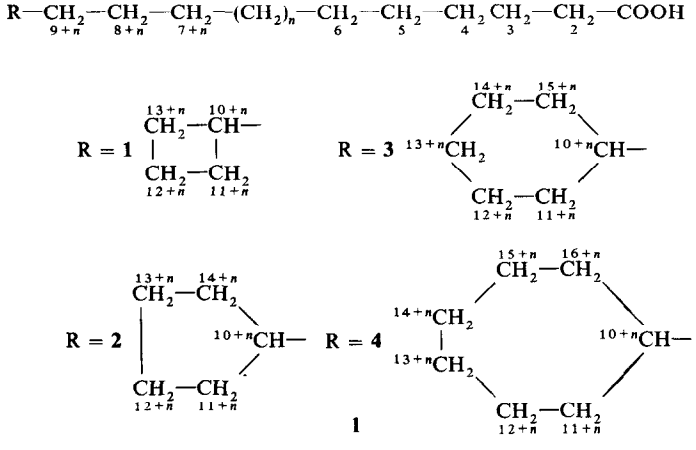
Chromatographic and spectroscopic data on these ω-cycloalkyl fatty acids were previously reported [6]. Apart from MS, the other spectroscopic techniques, IR and ^1H NMR have limited diagnostic value for distinguishing mixtures of such ω-cycloalkyl fatty acids. Preliminary work on ^{13}C NMR spectral analysis of fatty acids [7] shows the suitability of this technique for their structural analysis. The present paper deals with the capability of ^{13}C NMR to locate the cyclic group in a series of natural and semi-synthetic ω-cycloalkyl fatty acids.

As expected, the spectra of ω-cycloalkyl fatty acids, with the generalized formula **1**, show a n-fold accidental degeneracy (overlaps) due to the n-$\underline{CH_2}$-groups of nearly equivalent carbons at δ 29.65. The ^{13}C chemical shifts of the carbonyl of different ω-cycloalkyl fatty acids at δ 180.43 appear to be largely solvent dependent and in good agreement with the previously reported values [7]. The low intensity of this signal is due to the diminished nuclear Overhauser enhancement and long T_1 for the pulse conditions. The ^{13}C resonances of C-2 and C-3 in the carboxylic end group of the molecules (at δ 34.15 and 24.72, respectively) are easily assigned on the basis of well known chemical shift rules. The 0.2 δ differences in chemical shifts of C-4 (δ 29.10), C-5 (29.29) and C-6 (29.48) are in agreement with the predicted ^{13}C linear electric field effect shifts in saturated fatty acid chains. The assignments of the remaining carbon atoms at the end of the aliphatic chain for the ω-cyclobutyl, ω-cyclopentyl, ω-cyclohexyl and ω-cycloheptyl fatty acids are reported in Table 1. The ^{13}C resonance assignments of the carbon atoms C_{7+n}, C_{8+n} and C_{9+n} at the aliphatic chain end, respectively, γ, β and α to the cyclic group, are based on chemical shift rules [8, 9] and comparison with appropriate model compound [10, 11].

Table 1. ^{13}C Chemical shifts (from TMS $\pm$0.02) and multiplicities for the carbon atoms at the aliphatic chain end of ω-cycloalkyl fatty acids

Carbon No.	1	2	3	4
$7+n$ (*t*)	29.72	29.99	30.05	30.05
$8+n$ (*t*)	27.22	28.84	27.00	27.48
$9+n$ (*t*)	37.14	36.29	37.82	38.30
$10+n$ (*d*)	36.29	40.21	37.68	39.34
$11+n$ (*t*)	28.45	32.77	33.60	34.73
$12+n$ (*t*)	18.52	25.22	26.56	26.63
$13+n$ (*t*)	28.45	25.22	26.90	28.61
$14+n$ (*t*)		32.77	26.56	28.61
$15+n$ (*t*)			33.60	26.63
$16+n$ (*t*)				34.73

As expected, the cyclobutane and cyclopentane rings give one methine and two methylene signals with an intensity ratio of *ca* 1:1:2 for cyclobutane and *ca* 1:2:2 for cyclopentane. The cyclohexane and cycloheptane rings give one methine and 3 methylene signals with an



1

intensity ratio of *ca* 1:1:2:2 and 1:2:2:2, respectively. The assignments were confirmed by selective proton-decoupling experiments.

EXPERIMENTAL

Precursor acids were purchased commercially.

Large scale recovery of acids. Bacillus acidocaldarius was utilized as a source of ω-cycloalkyl fatty acids. The growth conditions, recovery and purification of natural and semi-synthetic ω-cycloalkyl fatty acids were as previously described [4].

^{13}C *NMR measurements.* All measurements were made with a Fourier transform accessory and signal multiplicity was determined by off-resonance decoupling. The samples of ω-cycloalkyl fatty acids (50 mg) were spun in 5 mm tubes using $CDCl_3$ (0.5 ml) as solvent. The solvent deuterium provided the lock signal; chemical shifts are given as δ values in ppm downfield from the internal TMS ^{13}C signal. Chemical shifts are accurate to within ± 0.02 ppm. Spectra were first recorded in the proton noise decoupling mode to measure chemical shifts and the degree of substitution of each carbon atom was then determined by obtaining a second series of spectra in the single frequency off-centre decoupling mode.

Acknowledgements—The authors thank Enrico Esposito and Salvatore Sodano for technical assistance.

REFERENCES

1. De Rosa, M., Gambacorta, A., Minale, L. and Bu'Lock, J. D. (1972) *Biochem. J.* **129**, 751.
2. Oshima, M. and Ariza, T. (1975) *J. Biol. Chem.* **250**, 6963.
3. Hansen, R. I. (1967) *Chem. Ind.* **39**, 1640.
4. De Rosa, M., Gambacorta, A. and Bu'Lock, J. D. (1974) *Phytochemistry* **13**, 905.
5. Dreher, R., Poralla, K. and Koning, W. R. (1976) *J. Bacteriol.* **127**, 1136.
6. De Rosa, M. and Gambacorta, A. (1975) *Phytochemistry* **14**, 209.
7. Batchelor, J. G., Cushley, R. J. and Prestegard, J. H. (1974) *J. Org. Chem.* **39**, 1698.
8. Stothers, J. N. (1972) *Carbon-13 NMR Spectroscopy.* Academic Press, New York.
9. Levy, G. C. and Nelson, G. L. (1971) *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists.* Wiley-Interscience, New York.
10. Breitmaier, E., Hess, G. and Voelter, W. (1975) *Atlas of Carbon-13 NMR Data.* Heyden, London.
11. Johnson, L. F. and Jankowshy, W. C. (1972) *Carbon-13 NMR Spectra.* Wiley-Interscience, New York.

Phytochemistry, 1979, Vol. 18, pp. 1736–1737. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1001-1736 $02.00/0

NEUE ACETYLENVERBINDUNGEN AUS *ATHANASIA TRIDENS**

FERDINAND BOHLMANN und CHRISTA ZDERO
Institut für Organische Chemie der Technischen Universität Berlin, Straße des 17. Juni 135, D-1000 Berlin 12, W. Germany

(*Eingegangen am* 19 *Februar* 1979)

Key Word Index—*Athanasia tridens*; Compositae; new C_{17} enediynene; new C_{18} acetylenic esters.

Die bisherigen Untersuchungen von Vertretern der südafrikanischen Gattung *Athanasia* (Tribus Anthemideae) haben gezeigt, daß eine Gruppe durch Furanesquiterpene und eine zweite durch Acetylenverbindungen, die auch in der nahe verwandten Gattung *Pentzia* vorkommen, charakterisiert werden kann [1–4].

Wir haben jetzt *A. tridens* Oliv. untersucht. Die Wurzeln enthalten **2**, **4**, **6**–**9** sowie die Methylester **1** und **3**. Die entsprechenden Säuren **2** und **4** haben wir bereits früher isoliert und in die Ester **1** und **3** übergeführt [1], so daß die Strukturen klar sind. Schließlich isoliert man noch das Acetat **5**, dessen Konstitution aus den spektroskopischen Daten folgt. Das UV-Spektrum zeigt, daß ein Endiinen vorliegen muß, während das ^1H-NMR-Spektrum die Konfigurationen der Doppelbindungen erkennen läßt. Offenbar wird **5** in Analogie zu ähnlichen Verbindungen durch β-Oxydation und Fragmentierung aus **12** über **2** gebildet (s. Schema). Die oberirdischen Teile liefern evenfalls **1**–**4**, **6**, **7**, **9** sowie Germacren D (**10**) und Cumarin.

Die Inhaltsstoffe dieses Vertreters zeigen, daß auch diese Art in die Gruppe der mit *Pentzia* nahe verwandten gehören dürfte.

EXPERIMENTELLES

IR: Beckman IR 9, CCl_4; ^1H-NMR: Bruker WH 270; MS: Varian MAT 711, 70 eV, Direkteinlaß. Die lufttrockenen zerkleinerten Pflanzenteile extrahierte man mit Ether–Petrol, 1:2 und trennte die erhaltenen Extrakte zunächst grob durch SC (Si gel, Akt. St. II) und weiter durch mehrfach DC (Si gel GF 254). 30 g Wurzeln ergaben in der Reihenfolge ihrer Polarität 2 mg **9**,

* 254. Mitt. in der Serie "Polyacetylenverbindungen"; 253. Mitt.: Bohlmann, F., Zdero, C., Robinson, H. und King, R. M. (1979) *Phytochemistry* **18**, 1519.