Short Communication

Haphazard Isolation of a Peroxide from Diethyl Ether, Autoxidation of Diethyl Ether and Structure of *Mozuku* Toxin A

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During an attempted isolation of alkaloids from ergot-infested smooth meadow grass a sample was extracted with unpurified diethyl ether from a laboratory stock bottle. After drying over sodium sulfate, evaporation of 150 ml of ether left 1.2 g of colorless oil of low viscosity. Subsequent repetitions of this experiment using doubly distilled diethyl ether or dichloromethane failed to yield this product, signifying that the oil was actually an impurity in the unpurified diethyl ether.

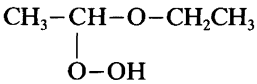
Further purification of the oil was effected by distillation at room temperature and $5 \cdot 10^{-3}$ mmHg. The purified sample exhibited *m/z* 88 as the highest mass peak in the mass spectrum. Peak matching revealed this peak to correspond to the composition $C_4H_8O_2$ (presumably ethyl acetate, either contained in the sample or formed as a degradation product in the inlet system of the mass spectrometer). Elemental analysis gave C 45.14 and H 10.15 %, compatible with the composition $C_4H_{10}O_3$ (calc. C 45.28, H 9.43 %), taking into account that the sample exploded violently in the combustion tube. Off-resonance and noise-decoupled ^{13}C NMR spectroscopy revealed the presence of four different carbon atoms, viz. two methyl groups appearing at δ 15.2 (q) and 18.1 (q) ppm, a methylene group at δ 64.2 (t) and a methine group at δ 104.3 (d) ppm. The signals of the protons associated with these carbon atoms are listed in Table 1. Extensive decoupling experiments confirmed the relationship expected from the coupling constants for the protons. The IR spectrum of the neat liquid exhibited strong absorptions at 3300 (OH), 2970 (aliphatic C–H),

Table 1. ^1H NMR spectral data for **1** and **2** in $CDCl_3$ (δ/ppm).

	$CH_3(-CH_2)$	CH_2	CH	CH_3	OH
(1-Ethoxyethyl)hydroperoxide (**1**)[a]	1.30 (m)[d]	3.83 (m)	5.10 (q)	1.30 (m)	9.95 br s
Oily product	1.24 (t)[e]	3.70 (dq)[f] 3.87 (dq)[f]	5.04[g]	1.35 (d)[g]	9.56 br s
1-(Ethylperoxy)ethanol (**2**)[b]	1.0 (t)	4.10 (q)	5.35 (q)	1.2 (d)	
Toxin A[c]	1.25 (t)[e]	3.69 (dq)[f] 3.85 (dq)[f]	5.04 (q)[h]	1.37 (d)[i]	8.56 br s

[a]Ref. 2. [b]Ref. 3. [c]Ref. 4. [d]J = 6 Hz. [e]J = 7 Hz. [f]J = 16 and 7 Hz. [g]J = 5.6 Hz. [h]J = 5 Hz. [i]J = 5.4 Hz.

1380, 1130 and 1100 cm^{-1}. A medium intensity band was also observed at 845 cm^{-1}, consistent with a hydroperoxide grouping.[1] Furthermore, the oil gave rise to rapid release of iodine on contact with an acidified iodide solution. These data leave only two possibilities for the structure of the oily compound, namely either (1-ethoxyethyl)hydroperoxide (**1**) or 1-(ethylperoxy)ethanol (**2**).



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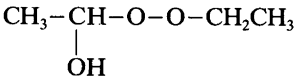

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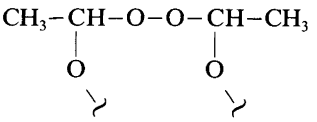
An inspection of the ^1H NMR data in Table 1 reveals that the oil must be (1-ethoxyethyl)hydroperoxide (**1**).

Comparison of the data in Table 1 leaves little doubt that the oily substance is identical to toxin A isolated from a brown alga, *Sphaerotrichia divaricata*.[4] The identity of the two compounds was further substantiated by the superimposability of the ^{13}C NMR spectra and the almost identical IR spectra of the two samples. Also, toxin A exhibited no molecular ion peak in the mass spectrum. Compounds similar to toxin A were found also in the brown algae *Cladosiphon okamuranus* and *Analipus japonicus,* and in the red alga *Gracilariopsis chorda.* These toxins are believed to be responsible for *Mozuku* poisoning, a human intoxication resulting from consumption of these brown algal species.[5] Toxin A is lethal to mice at a dose level of 250 μg g^{-1}. Since toxin A and the oily product have identical spectroscopic properties, the structure of toxin A should be revised from the originally proposed **2** to (1-ethoxyethyl) hydroperoxide (**1**).

Another compound, toxin B, was assigned[4] the partial structure **3** on the basis of ^1H NMR [δ 1.43



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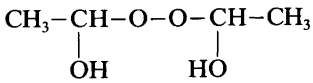

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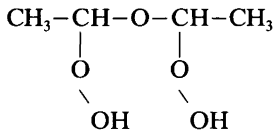
(6 H, d, J = 5.4 Hz) and 5.29 (2 H, q, J = 5.4 Hz) ppm] and ^{13}C NMR [δ 18.5 (q) and 104.0 (d) ppm] spectroscopic evidence. Furthermore, the principal toxin of another collection of *S. divaricata* was identified as bis(1-hydroxyethyl)peroxide (**4**) by IR, ^1H NMR [δ 1.43 (6 H, d, J = 5 Hz), 5.42 (2 H, q, J = 5 Hz) and 8.72 (2 H, br s) ppm] and by comparison with a synthetic sample [^1H NMR: δ 1.39 (6 H, d, J = 5 Hz), 5.35 (2 H, q, J = 5 Hz) and 9.73 (2 H, br s) ppm; ^{13}C NMR: δ 14.7 (q) and 108. 2 (d) ppm]. Differences in ^1H NMR and IR spectra were ascribed to differences in stereochemistry between the natural and synthetic toxins. It is possible that the latter toxin is actually α,α′-bis-hydroperoxy diethyl ether (**5**).



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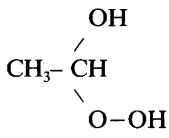

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Toxin B may in fact be the peroxyhydrate of acetaldehyde (**6**). The above considerations certainly warrant a reinvestigation of the *Mozuku* toxins.

The currently accepted mechanism for the photochemical autooxidation of diethyl ether accounts for the formation of (1-ethoxyethyl)hydroperoxide (**1**) and hydrogen peroxide. Although there is hardly any doubt about the structure of this oxidation product, this is, to our knowledge, the first time it has been isolated from diethyl ether and rigorously identified. The photochemical oxygenation of diethyl ether has been extensively investigated, resulting in the identification of several products of the initially formed hydroperoxide.[6,7] However, (1-ethoxyethyl)hydroperoxide (**1**) and hydrogen peroxide

seem to be the only peroxides identified, even though simple TLC experiments easily demonstrate the presence of at least three organic peroxides as well as hydrogen peroxide in freshly distilled diethyl ether shortly after exposure to sunlight.[8]

That the peroxide isolated in this study is actually identical to the primary peroxide formed in the autooxidation of diethyl ether was supported by a series of GC-MS studies (VG Masslab VG 20–250 quadrupole mass spectrometer, 20 m OV-101 fused silica gel column, temperature 40–100 °C, 10 °C min^{-1}). Injection of a sample of aged diethyl ether produced, apart from peaks corresponding to the well-known impurities and decomposition products, such as ethyl acetate (M^+ at *m/z* 88), a reproducible gas chromatographic peak giving intense signals at *m/z* 91 (~60 %), 73 (100 %), 63 (~20 %) and 61 (~90 %). The molecular ion (*m/z* 106) was not detected, but the three most intense signals were tentatively assigned to $[EtOCH{=}O{-}OH]^+$ ($M^+ - CH_3$, *m/z* 91), $[EtO{=}CHCH_3]^+$ ($M^+ - O_2H$, *m/z* 73), and $[CH_3CH{=}O{-}OH]^+$ ($M^+ - OEt$, *m/z* 61). This peak was absent in the gas chromatogram of a sample of freshly purified diethyl ether. However, after exposure to air and sunlight for about 30 min, the sample exhibited a small fraction with mass spectrum superimposable with the above. Concomitant with the development of this fraction, TLC (silica gel, $CHCl_3$/MeOH, 95/5) revealed the presence of a faint spot with R_f 0.45–0.55, identical to the major fraction of aged diethyl ether. We therefore agree that **1** is the primary autooxidation product but wish to emphasize that at least two other peroxides are formed concomitantly or subsequently. These structures need to be clarified before any detailed understanding of the mechanism of this reaction can be claimed.

References

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