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BIOLOGICALLY ACTIVE SULPHUR COMPOUNDS FROM THE GREEN ALGA *CHARA GLOBULARIS*

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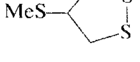
Key Word Index—*Chara globularis*; Charales; inhibitors of photosynthesis; sulphur heterocycles; 4-methylthio-1,2-dithiolane; 5-methylthio-1,2,3-trithiane.

Recently, several naturally occurring low molecular weight sulphur compounds have been shown to play important ecological roles; as scent markers [1], insecticides [2], and as inducers of feeding response behaviour [3]. Many other substances have been isolated and characterized without investigation of their potential biological effects [4].

During a study of the chemical ecology of some freshwater plants including the stoneworts (brittleworts, order Charales), our attention focused on *Chara globularis* Thuillier 1799 syn. *Chara fragilis* Gesvaux in Loiseleiur-Deslongchamps 1810. From an ecological point of view, this species is interesting since whenever it occurs in a pond it dominates the algal flora of the ecosystem. Furthermore, a rank, pungent smell is always associated with this plant. Initial experiments have revealed the presence of extremely active inhibitors of photosynthesis as determined in laboratory cultures of the unicellular green alga *Nitzschia palea* (Kütz) W. Smith [5].

We now wish to report the isolation and structure elucidation of two compounds which are at least partially responsible for the inhibition of photosynthesis and the characteristic odour of *C. globularis.*

Samples kept at −20° between collection and investigation were subjected to gas phase isopentane extraction in a modified Likens–Nickerson extractor [6] (sample size *ca* 1 kg wet wt corresponding to *ca* 150 g of dry wt). Gas entrainment of the resulting soln of volatiles gave rise to a mixture of compounds (50–100 mg), GC–MS analysis of which demonstrated the presence of two major sulphur-containing species. Column chromatography (Merck Si gel, eluent CH_2Cl_2–hexane, 1:4) afforded 2 fractions (5-12 mg each), homogenous on TLC and with the same retention times as those originally observed in the GC–MS experiment. The most volatile constituent according to high resolution MS has the elemental composition $C_4H_8S_3$ (**1**) (calc.: 151.1983, obs. 151.1977), while the less volatile compound contains an additional sulphur atom $C_4H_8S_4$ (**2**) (calc.: 183.9509, obs. 183.9518). ^{13}C NMR data for **1** indicate a symmetrical structure containing a methylthio grouping, since only 3 signals appeared, namely at δ 14.59 (SMe), 43.96 (SCH_2) and 50.90 (SCH) ppm with relative intensities 1:2:1, respectively. This hypothesis was substantiated by ^1H NMR data. The 90 MHz spectrum of **1** recorded in C_6D_6 exhibits a singlet at δ 1.51 (3H) and a multiplet at δ 2.4–2.8 ppm (5H), which at 270 MHz separated into two multiplets, one centred at δ 2.6 (1H) and the other at δ 2.8 ppm (4H), respectively. We therefore formulate **1** as 4-methylthio-1,2-dithiolane in agreement with the UV absorption band at 332 nm, characteristic of the 1,2-dithiolane ring system [7]. A pronounced solvent dependency of the ^1H NMR signals was observed, since the 90 MHz values for a $CDCl_3$ solution were shifted to δ 2.25 ppm (*s*, 3H) and δ 3.0–3.7 ppm (*m*, 5H).



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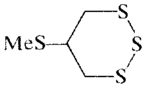

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The UV spectrum of **2** (λ_{max} 218 and 266 nm, hexane) is characteristic of the 1,2,3-trithiane ring (e.g. 5-hydroxy-1,2,3-trithiane has λ_{max} 210 and 265 nm [8]). The presence of the methylthio group was inferred from the singlet at δ 2.17 (3H, 270 MHz, $CDCl_3$) and the ^{13}C signals at δ 13.75 ppm. The ring proton appeared as multiplets centred around δ 3.24 and 3.05 (total of 5H) ppm, while the corresponding carbon atoms gave rise to signals at δ 42.65 and 45.51 ppm, respectively. We thus assign the structure of 5-methylthio-1,2,3-trithiane (**2**) to the less volatile compound.

Both compounds are thermolabile, **1** polymerizing and **2** loosing sulphur to form **1**, as evidenced by the possibility of retrieving the mass spectrum of **1** in the total elution volume between the two peaks representing **1** and **2** in the GC–MS analysis. The physiological effects of **1** and **2** are at present being further investigated as is the seasonal variation of activity in *C. globularis* and other members of Charales.

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PHYTUBEROL FROM *NICOTIANA RUSTICA* INOCULATED WITH TOBACCO MOSAIC VIRUS

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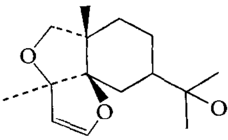
Key Word Index—*Nicotiana rustica*; Solanaceae; tobacco; sesquiterpenoid stress compound; phytuberol; tobacco mosaic virus.

Many structurally-related sesquiterpenoid stress compounds, such as rishitin and lubimin, have been isolated from the Solanaceae [1, 2]. Capsidiol [3], solavetivone [4, 5], phytuberin [6, 7], phytuberol [7] and glutinosone [8] have been shown to be stress compounds in plants of the genus *Nicotiana*. The first four compounds have been produced in leaves of *Nicotiana tabacum* in response to tobacco mosaic virus (TMV) [4, 5], tobacco necrosis virus (TNV) [3], the bacterium *Pseudomonas lachrymans* [6] or ethrel [7]. Capsidiol has also been found in leaves of *Nicotiana clevelandii* [3] and glutinosone in *N. glutinosa* inoculated with TMV.



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Here, we report the accumulation of terpenoid stress compound phytuberol (**1**) in leaves of *N. rustica* inoculated with TMV. The CH_2Cl_2 extract from the inoculated leaves contained phytuberol (**1**), which was first suggested by GC–MS analysis. The extract was steam-distilled and subsequently chromatographed on silicic acid column. Phytuberol was isolated from fractions eluted with hexane–Et_2O (1:1) and 100% Et_2O. The concentration of isolated phytuberol was 1.11 mg/g dry wt of inoculated leaves. Mass, IR and ^1H NMR spectra, $[\alpha]_D$ and the retention time of GLC of the isolated phytuberol were in accord with those of an authentic sample [9]. Phytuberol was not detected in healthy leaf by GC–MS analysis

EXPERIMENTAL

Nicotiana rustica was grown in a greenhouse at 24°. Tobacco plants (2 months old) with 6–8 fully expanded leaves were inoculated with TMV (0.5 μg/ml in 0.1 M phosphate buffer). Carborundum was used to aid virus infection. When brown lesions had been produced (7 days), the leaves were harvested.

The harvested leaves (396 g fr. wt) were frozen at $-20°$ for 2 days and then freeze-dried. The dried materials (45 g) were extracted with CH_2Cl_2 (1 l. × 3). The solvent was distilled off from the crude extract, which was then steam-distilled. The distillate (1.5 litre) was saturated with NaCl and extracted with Et_2O. The extract was evapd to dryness to give 230 mg of the volatile. The volatile was introduced onto a column of silicic acid (10 g) and eluted with hexane–Et_2O.

Phytuberol (**1**) was isolated as an oil (50 mg) from fractions eluted with hexane–Et_2O (1:1) and 100% Et_2O. Characteristic retention time on GLC was 10.3 min (5% OV 101 on Chromosorb W (AW), 3 mm × 1 m, 100–240°, 5°/min, 60 ml He/min).

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