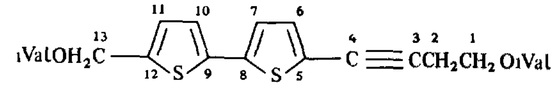

1

Scheme 1

(2H) and a triplet at δ4.25 (2H) further showed that these ester groups are primary. The chemical shifts agreed well with those of the corresponding diacetate which was isolated from a *Porophyllum* species [4]. The other signals also were nearly identical with those of the diacetate except those of the ester part. In addition to **1**, traces of other diesters were present which, however, could not be separated from senecioyloxymethylene terthienyl [2]. The aerial parts also gave **1** as well as the angelate of 5'-hydroxymethylene-2-(but-3-en-1-ynyl)-dithiophene [2], stigmasterol and sitosterol.

EXPERIMENTAL

The air-dried plant material (voucher deposited in RUBL Herbarium) was extracted with Et_2O–petrol (1:2) and worked-up in the usual fashion [5]. CC separation of the extract of 70 g roots gave a fraction (Et_2O–petrol, 1:4) which, by TLC (Et_2O–petrol, 1.20, several developments) finally gave, in addition of the esters reported previously [2] 3 mg 5'-isovaleryloxymethylene-2-(4-isovaleryloxy-but-3-inyl)-dithiophene (**1**), yellow oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO_2R); UV $\lambda_{max}^{Et_2O}$ nm: 335; MS *m/z* (rel. int.). 432.142 (1.3) (calc. for $C_{23}H_{28}O_4S_2$: 432.142), 330 $[M-RCO_2H]^+$ (9), 229 $[330-RCO_2]^+$ (11), 85 $[C_4H_9CO]^+$ (80), 57 $[85-CO]^+$ (100); CIMS: 433 $[M+1]^+$ (3), 331 $[M+1-RCO_2H]^+$ (100); ^{1}H NMR ($CDCl_3$, 400 MHz, TMS as int. standard): 4.25 (*t*, H-1), 2.79 (*t*, H-2), 7.02 (*d*), 7.00 (*d*), 6.97 (*d*), 6.96 (*d*, H-6, H-7, H-10, H-11), 5.21 (*s*, H-13), 2.22 and 2.23 (*d*), 2.12 (*m*), 0.95 (*d*), 0.91 (*d*) (O*i*Val) [*J* (Hz): 1, 2 = 7; 6, 7 = 10, 11 = 3.5; O*i*Val: 2', 3' = 3', 4' = 3', 5' = 7]. The aerial parts (250 g) gave 2 mg **1**, 5 mg 5'-angeloyloxy-methylene-2-(but-3-en-1-ynyl)-dithiophene, 50 mg stigmasterol and 25 mg sitosterol. Known compounds were identified by comparing with authentic material (^{1}H NMR, TLC).

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CO-OCCURRENCE OF Δ^5- AND Δ^7-STEROLS IN THE FUNGUS *DICTYUCHUS MONOSPORUS**

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Key Word Index—*Dictyuchus monosporus*; Saprolegniaceae; Oomycetes; fungi; sterols.

Abstract—The desmethyl sterol composition of the oomycete *Dictyuchus monosporus* is unusual in that it is a mixture of 56.9% Δ^5-sterols and 42.6% Δ^7-sterols. The Δ^5-sterols are cholesterol, 24 methylenecholesterol and fucosterol; the Δ^7-sterols are cholest-7-enol, ergosta-7,24(28)-dienol and stigmasta-7,*E*-24(28)-dienol. Stigmasta-7,*E*-24(28)-dienol is identified for the first time from natural sources. In addition, traces of lanosterol are present.

INTRODUCTION

The oomycetes have long been recognized as having sterol compositions different from the more advanced fungal groups. Most fungi contain ergosterol [1–3] but the oomycetes have Δ^5-sterols [4, 5] similar to certain algal groups [6, 7]. More recently, some of the oomycetes have been reported to contain sterols of the ergostane series instead of, or in addition to, Δ^5-sterols [8, 9]. To date, no oomycete has been found to contain both Δ^5- and Δ^7-sterols.

The co-occurrence of significant amounts of both Δ^5- and Δ^7-sterols in other organisms is uncommon. It is well-

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established that some higher plants which contain Δ^5-sterols [10] often have small amounts of Δ^7-sterols which are presumed to be precursors. The few plant groups which contain Δ^7-sterols as the dominant sterols typically lack Δ^5-sterols, presumably due to a block in the sterol biosynthetic pathway [11]. Recently, the co-occurrence of both Δ^7-sterols (71 %) and Δ^5-sterols (29 %) was reported in the leaves of *Beta vulgaris* [12]. The seed oil of *Trichosanthes kirilowii* had similar amounts of Δ^5- and Δ^7-sterols [13]. In addition, small amounts of Δ^5-sterols have been reported in oils of plants with Δ^7-sterols as the main sterols [14]. The purpose of this paper is to report the presence of significant amounts of both Δ^5- and Δ^7-sterols in the oomycete *Dictyuchus monosporus.*

RESULTS AND DISCUSSION

Dictyuchus monosporus contains six desmethyl sterols (Table 1). Three of these are Δ^5-sterols and the other three are their Δ^7-counterparts. In addition, a small amount (0.6 %) of the total sterol is lanosterol. Cholesterol, 24-methylenecholesterol and fucosterol have been reported as major sterols in several oomycetes [4, 5, 9]. GLC and GC/MS of these sterols from *D. monosporus* verified their structure as reported previously [15].

The RR_t of cholest-7-enol (lathosterol) matched that of an authentic cholest-7-enol standard on both the GC columns used. Its molecular ion peak was at m/z 386 (100 %). Other intense peaks were at 371 (25 %), 351 (7 %), 273 (19 %), 255 (67 %), 246 (6 %), 229 (19 %) and 213 (24 %). This mass spectrum matched that of an authentic sample of cholest-7-enol from our laboratory. Ergosta-7,24(28)-dienol (episterol) has been identified in a zygomycete [16], an ascomycete [17, 18], several lichens [19] and corn pollen [20, 21]. Its RR_t on each column matched that calculated by methods previously described [22, 23]. The mass spectrum was identical to campesta-7,24(28)-dienol reported recently [21].

The mass spectrum of the final desmethyl sterol gave the molecular ion at m/z 412 (3 %). Other peaks were at 397 (4 %), 314 (41 %), 299 (5 %), 271 (100 %), 255 (10 %), 231 (5 %) and 213 (7 %). This compound was tentatively identified as stigmasta-7,*E*-24(28)-dienol and compared favourably with published mass spectra descriptions of stigmasta-7,*Z*-24(28)-dienol [24, 12], although it compared more favourably with the older data with respect to small peaks due to loss of water and loss of water with other fragments [24]. The mass spectrum of stigmasta-7,*E*-24(28)-dienol also was quite similar to published mass spectral data of ergosta-7,24(28)-dienol, as would be expected [21]. A positive identification of stigmasta-7,*E*-24(28)-dienol was made utilizing GC by previously used methods [15]. The 4,4-dimethylsterol, lanosterol, was present in small amounts (0.6 %) and is assumed to be a precursor of other sterols in the organism. Lanosterol has been identified as a sterol precursor in another oomycete, *Saprolegnia ferax* [15].

D. monosporus is unusual in having a mixture of Δ^5-sterols (56.9 %) and Δ^7-sterols (42.6 %). To date no other oomycete has been reported with this combination. The significance of this sterol composition is unknown at present, but it is presumed that *D. monosporus* has a need for both Δ^5- and Δ^7-sterols.

Table 1 Sterol composition of *Dictyuchus monosporus*

Sterol	% sample	Weight (μg/mg dry wt)
Δ^5-Sterols		
Cholesterol	4.2	0.07
24-Methylenecholesterol	13.8	0.23
Fucosterol	38.9	0.65
Sub-total	56.9	—
Δ^7-Sterols		
Cholest-7-enol	10.8	0.18
Ergosta-7,24(28)-dienol	7.2	0.12
Stigmasta-7,*E*-24(28)-dienol	24.6	0.41
Sub-total	42.6	—
Other sterols		
Lanosterol	0.6	0.01
Total	100.1	1.67

EXPERIMENTAL

Plant material. *Dictyuchus monosporus* ATCC 34931 was obtained from the American Type Culture Collection and maintained on PYG agar. Cultures were grown for 2 weeks on PYG (Cantino) broth.

Isolation and identification of sterols. Sterols were isolated by our usual methods utilizing alumina CC, silicic acid TLC and argentation TLC [15]. Identification was based on GC utilizing two different columns (SE-30 and OV-17) and GC/MS. Authentic standards of cholesterol, cholest-7-enol, 24-methylenecholesterol, fucosterol and lanosterol were used for comparative purposes in GC and GC/MS analyses. Electron impact (70 eV) mass spectra were obtained from a Finnigan-MAT model 4500 mass spectrometer; data were collected and processed on an Incos data system.

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STEROLS OF THE SENEGALESE BROWN ALGA *PADINA VICKERSIAE*

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Key Word Index—*Padina vickersiae*; Phaeophyceae; brown algae; cholesterol; fucosterol; seasonal variation.

Abstract—Contrary to all the brown algae already studied for sterol content the Senegalese *Padina vickersiae* contains a great amount of cholesterol, chiefly in the later stages of growth. The seasonal variation of the sterols is studied and compared to other Phaeophyceae belonging to the genus *Cystoseira*.

INTRODUCTION

Some species belonging to the genus *Padina* have been studied for antimicrobial and antifungal properties [1–6]. In the course of our investigation of the Senegalese algae we have been interested in the endemic *Padina* species, namely *P. vickersiae*. This alga is particularly abundant along the coast near Dakar. The investigation of the sterol content gave surprising results.

RESULTS AND DISCUSSION

The usual work-up for sterol purification, including digitonin precipitation, alkaline hydrolysis and silica gel chromatography, yielded the sterol fraction which was analysed by GLC and GC/MS. Cholesterol and fucosterol were the major sterols but 22-dehydrocholesterol, 24-methylenecholesterol, campesterol, stigmasterol and sitosterol were also identified. A sterol sample obtained from a batch of algae harvested during the year 1982 had a ratio of fucosterol to cholesterol of 40 : 50 (F : C < 1). This compares with some species of brown algae belonging to the genus *Cystoseira* (Table 1) in which the ratio F : C is always high, fucosterol being the major sterol for all the previously described Phaeophyceae. *P. vickersiae* is in fact the first exception with cholesterol as the major sterol. It was, therefore, interesting to study the seasonal variation of the sterol content of this alga. This seasonal variation was marked by an increasing yield of sterols during the growth of the alga (Table 1), as observed for most secondary metabolites in microorganisms, usually stored just before the resting phase. Another interesting fact was the decrease of the sterol content of *P. vickersiae* with depth, confirming the results observed for *Cystoseira zosteroides* (Table 1). The ratio F:C, ranged from 2.5 to 0.7, and was very low for a Phaeophyceae species and the ratio decreased with depth and with the season from March to July. A similar variation was observed for the Mediterranean *Cystoseira elegans*, but the F:C ratios ranged from 38 to 12.

Thus *P. vickersiae* is an exception in the Phaeophyceae, with unusually high amounts of cholesterol all the season round. Cholesterol is even the major sterol at the later stages of the growth. However, it is not a general feature of the genus *Padina*. Indeed *Padina arborescens* [7] has already been described with fucosterol as the major sterol; for the Mediterranean *Padina pavonia* we observed more than 90% of fucosterol (F : C = 19).

EXPERIMENTAL

Algal material. *Padina vickersiae* (Hoyt) was harvested at Fann Cape near Dakar (Senegal) at the beginning of the month from