EXPERIMENTAL

Algal material. Samples of *Halarachnion ligulatum* were harvested in June at Logonna Daoulas, near Brest, Brittany.

Extraction. The alga (20 g) was extracted with 600 ml 16% NaOH at 90° for 5 hr. Diatomaceous earth was added, the mixture filtered, and the polysaccharide recovered by addition of 2.5 vol isopropanol at 80°.

Hydrolysis of the polysaccharide and sugar analysis. The polysaccharide (1 g) in 0.5 M H_2SO_4 (5 ml) was hydrolysed for 12 hr at 90°. The hydrolysate was neutralized, the neutral sugars reduced with $NaBH_4$ and the resultant alditols acetylated with Ac_2O–C_5H_5N (1:1). The alditol acetates were analysed by GC: glass column (1.8 mϕ1/8) containing 3% SP 2340 on supercoport (100/200 mesh); 210°; N_2 30 ml per min. Inositol acetate was used as the internal standard.

Sulphates was determinated by a turbidometric method [4]. The 3,6-anhydrogalactose residues were analysed by the resorcinol method using fructose for the standardization [5].

The total sugar content was measured by the phenolsulphuric method [6]. IR spectroscopy was performed on films prepared by evaporating (60°) 0.25% solns of the carrageenans on polyvinyl chlorate plates (Afco dur) ^{13}C NMR: 22.6 MHz, 30 mg/ml in D_2O at 95°, relative to int. DSS and converted to external TMS.

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STEREOCONFIGURATION OF SEQUOYITOL BY HIGH RESOLUTION ^{1}H NMR

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Key Word Index—*Podocarpus sellowii*, Podocarpaceae; sequoyitol; 5-methoxy-*myo*-inositol; *myo*-inositol.

Abstract—The stereoconfiguration of sequoyitol, isolated as the pentaacetate from the leaves of *Podocarpus sellowii*, has been established by comparing its ^{1}HNMR spectrum with that of *myo*-inositol hexaacetate.

INTRODUCTION

Because of very poor solubility in common organic solvents and of extreme polarity, degradative methods have always been used for establishing the stereochemistry of the inositols. However, it has been shown recently [1] that high resolution ^{1}H NMR spectroscopy can be successfully exploited for elaborating the stereoconfigurations of the inositols by using the respective acetates as starting materials.

RESULTS AND DISCUSSION

In continuation of our work in this field [1], sequoyitol was isolated by dehydrating the water extract of the hydrophilic portion of the total ethanol extract of the leaves of *Podocarpus sellowii* after separating the diterpenes [2]. The gummy material upon acetylation followed by usual work-up furnished the pentaacetate (**1a**), $C_{17}H_{24}O_{11}$ mp 200°, m/z 404 $[M]^+$, which showed in the IR spectrum peaks for acetoxy and methoxy functions at 1760, 1430, 1370 and 1235 cm^{-1} besides the characteristic absorptions in the finger print region at 1168, 1140, 1094, 1065, 951 and 912 cm^{-1}, typical for sequoyitol pentaacetate as was reported in ref. [3]. The integration of all the resonance intensities in the 360 MHz ^{1}H NMR spectrum, as well as the resonance pattern, suggests that the three intense singlets at δ 2.19 (3H), 2.08 (6H) and 1.99(6H) are assigned for the five acetoxy groups, the former is axial and the latter two for four acetoxy functions, all equatorial. The other intense singlet at δ 3.45 represents the methoxyl protons. The two pairs of doublets at 4.99 and 5.02 ($J = 2.80$ Hz) are for the two axial

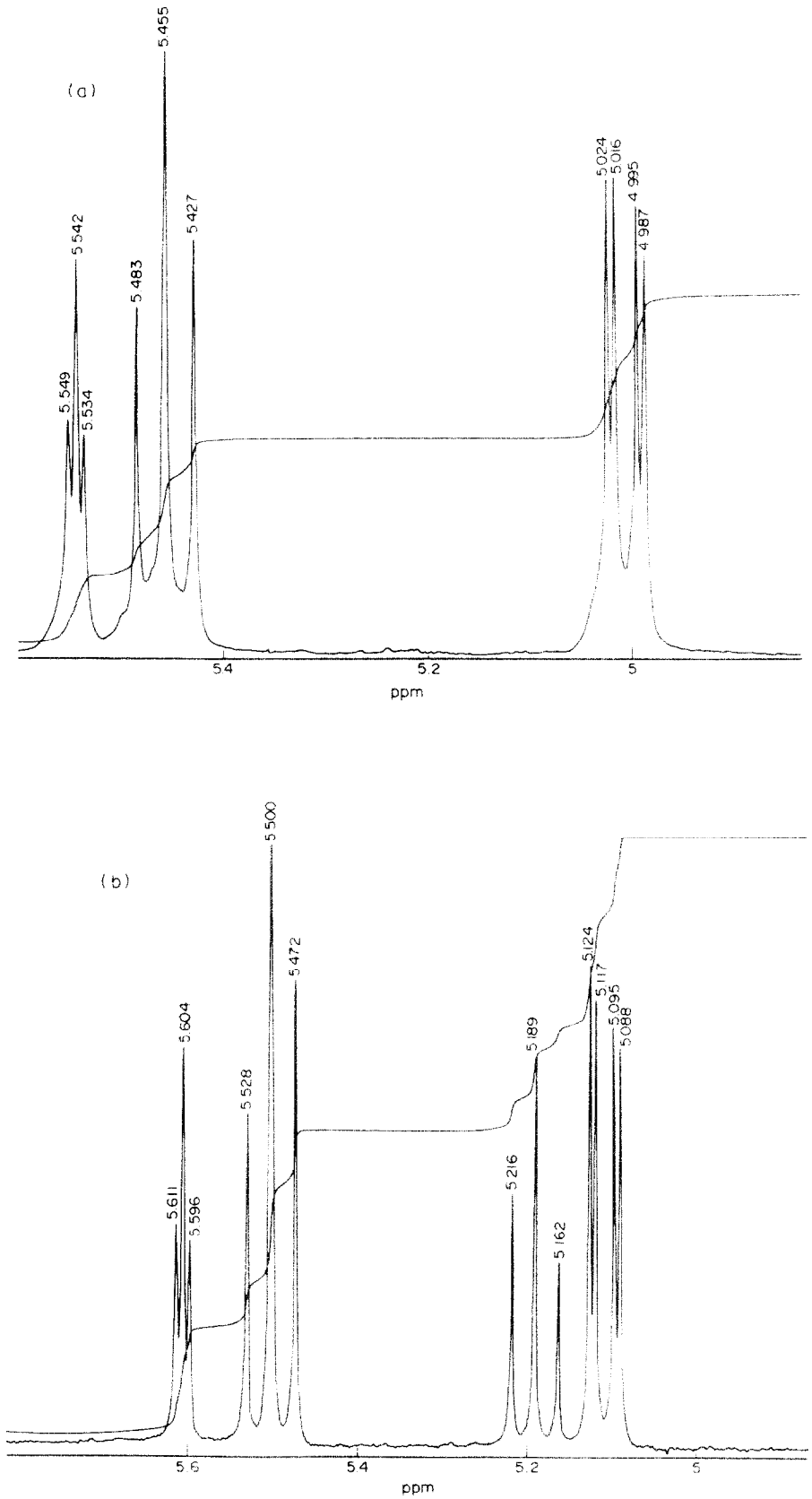
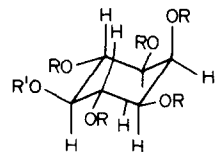


Fig. 1. 360 MHz ^{1}H NMR spectra of the methine portions of (a) sequoyitol (5-methoxy-*myo*-inositol) pentaacetate (**1a**) and (b) *myo*-inositol hexaacetate (**1d**).



	R	R'
1a	Ac	Me
1b	H	Me
1c	R'	H
1d	R'	Ac

methine protons at C-1 and C-3 whereas the downfield triplets centered at 5.45 ($J = 10.10$ Hz) and 5.54 ($J = 2.80$ Hz) are for the other two axial methine protons of C-4 and C-6 and the equatorial one of C-2 (Fig. 1a). The up-field triplet at 3.41 ($J = 10.10$ Hz) is for the remaining axial methine proton of C-5, the same carbon also bearing the methoxyl group.

The pentaacetate (**1a**) upon hydrolysis with dry ammoniacal methanol at low temperature gave sequoyitol (**1b**), $C_7H_{14}O_6$, mp 238-239°, *m/z* 195 $[M+1]^+$. Demethylation of **1b** with 57% aqueous hydriodic acid at reflux temperature gave *myo*-inositol (**1c**), $C_6H_{12}O_6$, mp 220°, which in the mass spectrum showed peaks at *m/z* (rel.int.) 181 $[M+1]^+$ (0.6), 163 (0.6), 144 (1.5), 102 (7.9), 73 (100, base peak) and 60 (5.5), in accordance with the peak positions and fragmentation scheme reported earlier [4]. Acetylation of **1c**, with acetic anhydride and dry pyridine by heating on a water bath gave the hexaacetate (**1d**), $C_{18}H_{24}O_{12}$, mp 214–215°. Besides the molecular ion peak at *m/z* (rel.int.) 432 $[M]^+$ (0.2), the mass spectrum of the hexaacetate **1d** showed a series of peaks at 390 (0.4), 373 (2.1), 330 (0.9), 252 (2.4), 241 (4.9), 210 (50.3), 168 (58.1) 157 (23.0), 126 (27.9), 115 (29.8), 73 (9.4) and 43 (100, base peak), i.e. the same values as reported earlier [4]. As expected, the 360 MHz ^{1}H NMR spectrum of the hexaacetate (**1d**) shows some significant differences with the spectrum of the pentaacetate (**1a**). However, the spectrum is extremely informative and self revealing. There are no methoxy and methine peaks around δ 3.45. The three acetoxy peaks at 2.21 (3H), 2.02 (9H) and 2.00 (6H) are, respectively for the C-2 axial, C-4, C-5 and C-6 equatorial and the other two equatorial acetoxyls at C-1 and C-3 on either side of the C-2 axial group. The down-field triplets at 5.60 (J = 2.80 Hz) and 5.50 (J = 10.10 Hz) are for the C-2 equatorial and C-4 and C-6 axial methine protons as was the case with the pentaacetate (**1a**). The two pairs of doublets at δ 5.09 and 5.12 (J = 2.80 Hz) are for the two axial methine protons of C-1 and C-3. However, a new triplet centered at 5.19 (J = 10.10 Hz) not present in the spectrum of the pentaacetate (**1a**), was assigned to the axial methine proton of C-5 (Fig. 1b). The down-field shift experienced by the methine proton of C-5 was due to the generation of the acetoxy function in the hexaacetate in place of the methoxyl as was in the pentaacetate.

Thus a very careful examination of the two 360 MHz ^{1}H NMR spectra of the pentaacetate (**1a**) of sequoyitol and the hexaacetate of *myo*-inositol (**1d**) clearly suggests the stereoconfiguration of sequoyitol as **1b**.

EXPERIMENTAL

NMR: $CDCl_3$ using TMS as int. standard. IR: $CHCl_3$ (6%).

Preparation of sequoyitol pentaacetate (**1a**). Air-dried finely ground leaves (2.3 kg) of *P. sellowii* Klotz., collected from Caruarú, Pernambuco, were extracted with 95% EtOH (9 l) for 24 hr in a Soxhlet. The conc. EtOH extract (0.3 kg) was then vigorously agitated with ice cold distilled H_2O (1.5 l) and filtered through celite with a Buchner funnel. The slightly turbid brown aqueous filtrate was then exhaustively extracted with hexane, C_6H_6, $CHCl_3$ and EtOAc (~4.5 l of each) removing all hydrophillic diterpenes [2]. It was then dried on a rotary evaporator under vacuum to a red brown gummy material A (0.10 kg). The gum A (40 g) upon acetylation with Ac_2O and pyridine according to the known procedure [1] gave sequoyitol pentaacetate (**1a**, 0.31 g), which was obtained in pure form after three crystallizations from C_6H_6–hexane in colourless needles, mp 202°, found to be identical in all respects with an authentic sample (mp,mmp, IR, NMR and mass).

Preparation of sequoyitol (**1b**), myo-*inositol* (**1c**) and myo-*inositol hexaacetate* (**1d**). Dry powdered **1a** (0.25 g) was hydrolysed following the technique of ref. [1] when sequoyitol was obtained (**1b**, 0.11 g) in pure form which was then crystallized from aq. EtOH in colourless needles, mp 238–239°, identical with an authentic sample [2] in all forms. Demethylation of **1b** (0.10 g) by known procedure [1] furnished *myo*-inositol (**1c**, 0.08 g), which was crystallized from aq. EtOH in colourless needles, mp 220°, identical with an authentic sample [1]. *Myo*-inositol (**1c**, 0.07 g) upon acetylation and work up [1] gave in quantitative yield *myo*-inositol hexaacetate (**1d**, 0.08 g) which was crystallized from $CHCl_3$–EtOH in colourless needles, mp 214–215°, and was identified with the authentic sample of ref. [1].

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