PC [*n*-BuOH–H_2O–HOAc (12:5:3), Schleicher & Schüll paper 2315, 1.8 mg material/cm, descending paper strip chromatography, 2 days; **1** migrated *ca* 20 cm; detection with ninhydrin] and ion exchange chromatography (Dowex 50 WX 8, 200–400 mesh, analytical grade, 0.1 M pyridine formate buffer pH 3.3, column 2.2 × 36 cm, 20-ml fractions). Fractions 15–24 gave 20 mg **1** from H_2O–EtOH with decomp. above 250° and $[\alpha]_D^{22} + 43.8°$ (H_2O, *c* 0.51). The IR spectrum (KBr) proved to be identical with that of (−)-nicotianamine.

(2R:3'R) - N - (3 - *Amino* - 3 - *carboxypropyl*) - *azetidine* - 2 - *carboxylic acid* (**2**). The residue of the Sephadex G-10 fractions 34–37 (306 mg, see above) was purified by ion exchange chromatography (conditions as above). Fractions 10–20 gave 68 mg needles from H_2O–EtOH with decomp. above 240° and $[\alpha]_D^{22} + 76.4°$ (H_2O, *c* 0.81). The IR spectrum (KBr) proved to be identical with that published for its antipode [5].

Biological test. Seedlings of *Lycopersicon esculentum* Mill. cv 'Bonner Beste' mutant *chloronerva* were raised in quartz sand and transferred to a nutrient soln after the first leaf became visible. Composition of the nutrient soln was: $Ca(NO_3)_2$ 5×10^{-3}; KNO_3 5×10^{-3}; KH_2PO_4 1×10^{-3}; $MgSO_4$ 1×10^{-3}; H_3BO_3 4.6×10^{-5}; FeEDTA 5×10^{-6} M. Plants were cultivated in a growth cabinet at a photoperiod of 16 hr light/8 hr dark; temp. 25/20°; r.h. 70 ± 5%; light intensity (photosynthetically active radiation 400–700 nm) 300–310 $\mu E/m^2$ per sec; lamp type: fluorescent tubes, 90% 'warm white', 10% 'Lumoflor' (VEB Narva, Berlin). The biological test was performed after dissolution of the respective substance in 0.05% Tween 20 (Atlas-Goldschmidt GmbH, Essen, West Germany) by painting the chlorotic leaflets 5× per day with a smooth brush. Each treatment was performed with five seedlings. The response was considered positive when a change of the chlorotic leaflets to a normal green colour was observed which happened at the lowest concn. used within 4 days.

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3-[(7Z)-HEXADECENYL]-4-METHYLFURAN-2,5-DIONE FROM *PIPTOPORUS AUSTRALIENSIS*

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Key Word Index—*Piptoporus australiensis*; Polyporaceae; basidiomycete; structural determination; citraconic anhydride derivative; 3-[(7*Z*)-hexadecenyl]-4-methylfuran-2,5-dione.

Abstract—The structure of a new citraconic anhydride derivative from *Piptoporus australiensis* is established by spectroscopic and chemical methods as 3-[(7*Z*)-hexadecenyl]-4-methylfuran-2,5-dione.

INTRODUCTION

Recently we reported [1] the isolation of several novel polyolefinic compounds which are responsible for pigmentation in the bright orange fruiting body of the basidiomycete *Piptoporus australiensis* (Wakefield) Cunningham. We describe here the isolation from the same fungus of a colourless metabolite to which we assign the substituted citraconic anhydride structure (**1**). This is the first reported occurrence of a citraconic anhydride derivative in a basidiomycete. Only two other organisms are known to produce monomeric* substituted citraconic anhy-

*The 'nonadrides', a group of compounds isolated from various *fungi imperfecti* [4] are derived by dimerization of short side-chain derivatives of citraconic anhydride [5].

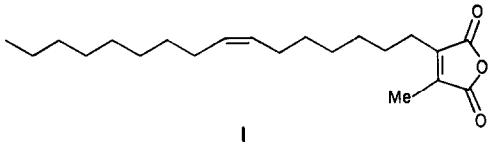
drides; *Aspergillus itaconicus* afforts itaconitin [2] and *A. wentii* produces four oxygenated long chain derivatives [3].

RESULTS AND DISCUSSION

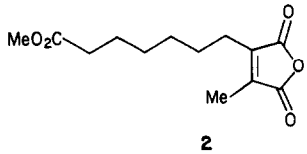
Preliminary separation of the crude Me_2CO extract of the fresh fungus by trituration with petrol followed by 'flash' chromatography [6] of the petrol soluble fraction gave **1** which was purified by distillation. The yield of pure **1** corresponds to 16% of the total Me_2CO extractives.

A molecular formula $C_{21}H_{34}O_3$ for **1** is indicated by MS and elemental analysis. An unsaturated cyclic anhydride nucleus, accounting for all three oxygen atoms in the molecule, is suggested by IR absorptions at 1860, 1825 and 1770 cm^{-1} [7] together with a UV maximum at 248.5 nm (log ϵ 3.74) and is confirmed by the presence in the ^{13}C NMR spectrum of quaternary carbon resonances at δ 165.8 and 166.2 (C=O) and at δ 140.4 and 144.7 (>C=C<) typical of a dialkyl substituted maleic anhydride [8]. The identity of one substituent as Me follows from examination of the ^1H NMR spectrum in which **1** exhibits a three proton singlet at δ 2.04 typical of a citraconic (methylmaleic) anhydride [3, 9]; the corresponding carbon resonance appears at δ 9.48. The ^1H NMR spectrum also identifies the hexadecenyl side chain of **1**. The protons of the CH_2 group adjacent to the anhydride nucleus appear as a triplet at δ 2.44 [3, 9]. Signals at δ 0.86 (*t*, 3H), δ 5.32 (*m*, 2H), δ 1.22–1.70 (*m*, 20H) and δ 1.98 (*m*, 4H) are assigned, respectively, to the protons of the terminal Me group, the double bond, and to ten in-chain and two allylic CH_2 groups. The position of the side-chain double bond was established by ozonolysis. Oxidative decomposition of the ozonide followed by methylation of the resulting carboxylic acids gave methyl nonanoate and a new citraconic anhydride derivative (**2**), $C_{13}H_{18}O_3$, the spectra of which (Experimental) are in full accord with the assigned structure.

Comparison of the remaining signals in the ^{13}C NMR spectrum of **1** with data for various (*Z*)- and (*E*)-octadecenoates [10, 11] and similar esters [12] permits assignment of the majority of ^{13}C resonances arising from the hexadecenyl side chain in **1** (Experimental) and allows the unambiguous assignment of (*Z*)-stereochemistry to the side-chain double bond. Of particular significance in this regard are the chemical shifts of the allylic carbons C-6′ and C-9′ (δ 27.1 and 27.5) which are characteristic of a (*Z*)-olefin of this type [10–12]. The allylic carbons of the corresponding (*E*)-olefin would be expected to appear near δ 32.5 [10–12].



1



2

The biosynthesis of **1** probably involves an initial condensation between C-2 of oleic acid and the C-2 carbonyl of oxalacetate [5] followed by decarboxylation and dehydration. The product, namely 3-[(7*Z*)-hexadecenyl]-4-hydroxy-5-methylene-2(5H)-furanone, of the alternative mode of condensation between C-2 of oleic acid and the C-1 carboxyl of oxalacetate has been isolated from higher plants [13].

EXPERIMENTAL

Extraction and isolation. Whole fresh sporophores (three specimens; 15–18 cm in diameter, *ca.* 6 cm in thickness) of *Piptoporus australiensis* (voucher specimen on deposit in the Herbarium of the Royal Botanic Gardens, Edinburgh under collection number WAT. HERB. 13878-E) collected at Marker Point, N.S.W., Australia, were chopped and immersed overnight in Me_2CO (1 l.) at room temp. After filtration and re-extraction (2×) the combined extracts were evaporated leaving a red gum (50 g). Trituration with petrol (3 × 500 ml) at room temp. and removal of the solvent from the combined extracts yielded a mobile red oil (16 g). A portion of this oil (550 mg) was applied to a column (4 cm) of Si gel which had been packed and was subsequently eluted with CH_2Cl_2 precisely as advocated by Still [6]. Evaporation of those fractions containing **1** (TLC Si gel HF_{254}; CH_2Cl_2) and distillation gave 3[(7*Z*)-*hexadecenyl*] - 4 - *methylfuran* - 2,5 - *dione* (**1**) (270 mg), an oil, bp 105°/0.1 mmHg (Kugelrohr)(Found: C, 75.0; H, 10.25; M^+, *m/z* 334.2512. $C_{21}H_{34}O_3$ requires C, 74.4; H, 10.25; M^+, *m/z* 334.2507).

IR ν_{max}^{film} cm^{-1}; 1860, 1825 and 1770 (O=C–O–C=O). UV λ_{max}^{hexane} nm (log ϵ): 248.5 (3.74). ^1H NMR (100 MHz, $CDCl_3$): δ 0.86 (3H, *t*, *J* = 6 Hz, H-16′), 1.22–1.70 (20H, *m*, $-CH_2-$), 1.98 (4H, *m*, H-6′ and H-9′), 2.04 (3H, *s*, C-4 Me), 2.44 (2H, *t*, *J* = 7 Hz, H-1′), 5.32 (2H, *m*, H-7′ and H-8′). ^{13}C NMR (15.04 MHz, $CDCl_3$): δ 9.48 (*q*, C-4 Me), 14.2 (*q*, C-16′), 22.7 (*t*, C-15′), 24.4 (*t*, C-1′), 27.1 and 27.5 (both *t*, C-6′ and C-9′), 28.8 and 29.3 (both *t*, C-2′ to C-5′ and C-10′ to C-13′), 31.9 (*t*, C-14′), 129.5 and 130.1 (both *d*, C-7′ and C-8′), 140.4 (*s*, C-4), 144.7 (*s*, C-3), 165.8 and 166.2 (both *s*, C-2 and C-5). EIMS (probe) 70 eV, *m/z* (rel. int.): 334 $[M]^+$ (31), 290 $[M-CO_2]^+$ (21), 289 (100), 191 (10), 181 (10), 177 (12), 168 (18), 163 (13), 151 (18), 150 (14), 149 (13), 126 (59), 109 (12), 98 (15), 97 (19), 95 (22), 84 (14), 83 (29), 82 (12), 81 (29), 79 (12), 71 (11), 70 (21), 69 (43), 68 (12), 67 (36).

Ozonolysis of **1**. A stream of ozone was passed through **1** (46 mg) in $CHCl_3$ at −70° until excess oxidant was detected (starch–KI paper) at the outlet. Excess ozone was removed with N_2 and the solution was warmed to room temp. and the solvent removed under red. pres. To the oily ozonide in Me_2CO (2 ml) at 0° was added an excess of Jones' reagent. After 0.5 hr *iso*-PrOH, then H_2O was added and the products were isolated with Et_2O. Methylation (CH_2N_2) and 'flash' chromatography (Si gel; 1 cm column; CH_2Cl_2) [6] gave methyl nonanoate (20 mg), which was identified by its ^1H NMR spectrum and computer-matched EIMS fragmentation pattern, and 3 - (6 - *methoxycarbonylhexyl*) - 4 - *methylfuran* - 2,5 - *dione* (**2**) (20 mg), an oil, bp 85°/0.1 mmHg (Kugelrohr) (Found: C, 61.8; H, 7.2. $C_{13}H_{18}O_5$ requires C, 61.4; H, 7.15%). IR ν_{max}^{film} cm^{-1}; 1855, 1825 and 1765 (O=C–O–C=O), 1740 (C=O). ^1H NMR (100 MHz, $CDCl_3$): δ 1.26–

1.80 (8H, *m*, $-CH_2-$), 2.07 (3H, *s*, C-4 Me), 2.32 (2H, *t*, $J = 7$ Hz, H-6'), 2.46 (2H, *t*, $J = 7$ Hz, H-1'), 3.66 (3H, *s*, OMe). EIMS (probe) 70 eV, *m/z* (rel. int.): 255 $[M+1]^+$ (6), 224 (10), 223 $[M-MeO]^+$ (80), 208 (24), 204 (17), 194 (20), 180 $[M-C_3H_6O_2]^+$ (15), 177 (25), 176 (14), 166 (13), 163 (12), 150 (45), 149 (29), 148 (14), 135 (14), 129 (29), 126 (68), 122 (10), 121 (17), 107 (14), 98 (26), 97 (41), 95 (13), 93 (17), 91 (11), 88 (11), 87 (21), 84 (16), 83 (12), 81 (21), 79 (26), 77 (13), 74 $[C_3H_6O_2]^+$ (100).

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WAX COMPOSITION OF *SARGASSUM FULVELLUM**

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Key Word Index—*Sargassum fulvellum*; Sargassaceae; Phaeophyta; wax; 5-methylhexyl esters; 2-ethylhexyl esters; 5-methylhexanol; 2-ethylhexanol.

Abstract—Sixty-seven compounds were characterized in the wax of *Sargassum fulvellum*. Characteristic components were the 5-methylhexyl esters of octanoic, decanoic, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic, and the 2-ethylhexyl esters of the same acids. The wax of *S. fulvellum* contains hydrocarbons (1.6%), esters (21.8%), free acids (74.9%) and free alcohols (0.3%). The principal free alcohols range in chain length only from C_6 to C_7.

INTRODUCTION

Sargassum fulvellum (Japanese name, 'Hondawara') is an annual seaweed which is used in folk medicine and for food, but the wax constituents have not so far been studied. Other waxes and constituents of the *Sargassum* genus have been studied: sargasterol from *S. ringgoldianum* [1], sarganan and sarganol from *S. natuns* [2], alginate and alginic acid from *S. swartzii*, *S. johnstonii* and *S. tenerrinum* [3], fucosterol and saringosterol from *S. ringgoldianum* [4]. In this paper, the wax components from *S. fulvellum* are reported.

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RESULTS AND DISCUSSION

The fronds of *S. fulvellum* were collected from the seashore in Kushikino-shi, Kagoshima, Japan, in August 1979. The dried alga was chopped finely and extracted with CH_2Cl_2 for 90 days at room tem-