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TWO POLYACETYLENIC PHYTOALEXINS FROM *ARCTIUM LAPPA**

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Key Word Index—*Arctium lappa*; Compositae; burdock; stress metabolites; phytoalexins; polyacetylenes.

Abstract—Treatment of sliced burdock root tissue with copper (II) sulphate stimulated phytoalexin formation. Two were isolated and characterized as (*S*)-12,13-epoxy-2,4,6,8,10-tridecapentayne and 1-tridecen-3,5,7,9,11-pentayne by spectroscopic methods and some chemical transformations.

INTRODUCTION

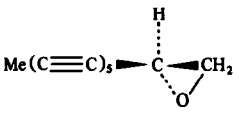
Although the Compositae is one of the biggest plant families and has been a rich source of secondary metabolites, few phytoalexins have been reported from this family [2]. Two polyacetylenes, safynol and dehydrosafynol [3, 4], and a coumarin ayapin [5] were recorded as phytoalexins of safflower (*Carthamus tinctorius*) and sunflower (*Helianthus annuus*), respectively. Recently, we reported two sesquiterpenoid phytoalexins, lettucenin A and costunolide from lettuce (*Lactuca sativa*) [6]. In a continuation of our search for composite phytoalexins we examined burdock *Arctium lappa* (gobo in Japanese), whose root is used as a food and a folk medicine in Japan. The present report describes the isolation and structure elucidation of two burdock phytoalexins **1** and **2** as (*S*)-12,13-epoxy-2,4,6,8,10-tridecapentayne and 1-tridecen-3,5,7,9,11-pentayne, respectively.

RESULTS AND DISCUSSION

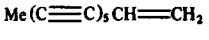
Treatment of sliced burdock roots with phytopathogenic bacteria, copper sulphate ($CuSO_4$), or UV irradiation induced the production of several antifungal compounds as evidenced by TLC bioassay. For preparative work, the slices were treated with 0.5% aq. $CuSO_4$ and then incubated for 3 days. The acetone extract from the incubated slices gave two extremely unstable compounds **1** and **2** after repeated chromatography on silica gel. The compounds **1** and **2** completely inhibited the conidial germination of *Bipolaris leersiae* at concentrations of 0.25 and 100 ppm, respectively.

The first compound **1**, $C_{13}H_6O$, $[\alpha]_D = +52.8°$ ($CHCl_3$), showed the UV spectrum characteristic of conjugated pentaynes [7] (see Experimental). Its ^1H NMR spectrum indicated the presence of one methyl group attached to a triple bond (δ2.00, 3H, singlet) and one terminal epoxide ring (δ2.96, 2H, *d*-like, $J = 3$ Hz; δ3.43, 1H, *t*-like, $J = 3$ Hz). Catalytic hydrogenation of **1** over PtO_2 afforded 1-tridecanol confirming **1** to be 12,13-epoxy-2,4,6,8,10-tridecapentayne. The absolute configuration of **1** was next examined. Catalytic hydrogenation of **1** using Pd on $BaSO_4$ gave a saturated epoxide **3b**, which gave a 2-tridecanol **4b** on lithium aluminium hydride reduction. The absolute configuration of (+)-1,2-epoxytridecane (**3a**) is known to be *R* [8]. Since the reported optical rotation of **3a** was so small [8] and the available amounts of the epoxide **3b** were limited, the alcohol **4b** was converted to a MTPA-ester **5b** (see Experimental) to compare it directly with the MTPA-ester **5a** derived from **4a**. Comparison of the both MTPA-esters (**5a** and **5b**) by HPLC and ^1H NMR spectroscopy indicated that **5b** was not identical to **5a**. Consequently, **1** should have the (12*S*)-configuration. Although 12,13-epoxy-2,4,6,8,10-tridecapentayne is a known polyacetylene [7] of composite plants, there has been no report on its absolute configuration.

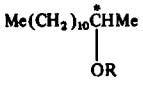
The second compound **2**, $C_{13}H_6$, showed spectral data (see Experimental) consistent with 1-tridecen-3,5,7,9,11-pentayne [7]. This compound has been found in many Compositae plants in very low concentrations [7]. Some species of *Arctium* [9] also contain **2**. Washino *et al.* reported recently eight polyacetylenes from *A. lappa* [10].



1



2



4a 2*S*, R = H
4b 2*R*, R = H
5a 2*S*, R = (*R*)-MTPA
5b 2*R*, R = (*R*)-MTPA

*Part 5 in the series 'Studies on Stress Metabolites'. For part 4 see ref. [1].

However, they mentioned the absence of **2** in the roots of the plant. We found that the amounts of **2** in the peeled control root tissue were negligible but increased 30 times in the peeled $CuSO_4$-treated root tissue. Interestingly, the concentration of **2** in the untreated epidermal root tissue was much higher than that of the inner control root tissue, suggesting some role for **2** as a plant-defence mechanism.

Acetylenic phytoalexins have been also reported from other plant families; furanoacetylenes from *Vicia* and *Lens* species (Leguminosae) [11], falcarindiol from carrot (Umbelliferae) [12] and tomato (Solanaceae) [13].

EXPERIMENTAL

^1H (100 or 400 MHz) NMR spectra and optical rotation were recorded in $CDCl_3$ and in $CHCl_3$, respectively. MS were measured with a direct inlet system at 70 eV.

TLC bioassay. Devloped silica gel sheets (Merck, Kieselgel 60 F_{254}; ether) were air-dried, sprayed with a dense conidial suspension of *Bipolaris leersiae* in potato-glucose medium, and incubated in a moist box at 25° for 2 days [1].

Induction and isolation of (S)-12,13-*epoxy*-2,4,6,8,10-*tridecapentayne* (**1**) *and* 1-*tridecen*-3,5,7,9,11-*pentayne* (**2**). Burdock roots, *A. lappa* L. cv Shirohada-sakigake, (30 kg) were washed, the epidermal tissue removed and cut into slices 2 mm thick. The slices were kept in moist boxes at 25° overnight and then treated with 0.5% aq. $CuSO_4$. After being incubated at 25° for 3 days, the slices, which became brown, were freeze-dried (2.7 kg) and extracted × 3 with Me_2CO. The combined extract was concd to 2.0 l and kept at 5° until used to avoid decomposition of sensitive active compounds. A one-tenth portion of the extract was evapd to leave 5.1 g of residue, which on silica gel CC using Et_2O as eluent gave 6 bioactive fractions. The least polar active fraction (1.2 g) was further sepd by sequential chromatography using silica gel (CH_2Cl_2–MeOH, 49:1), μ-Porasil (*n*-hexane–CH_2Cl_2, 2:1), and μ-Porasil (*n*-hexane) columns to give **1** (52 mg) and **2** (2 mg).

(S)-12,13-*Epoxy*-2,4,6,8,10-*tridecapentayne* (**1**). Pale yellow crystals, decomposing immediately and becoming violet to black in colour; high resolution MS, *m/z* 178.04375 $[M]^+$ (calc. for $C_{13}H_6O$: 178.04357); EIMS, *m/z* (rel. int.): 178 (82), 162 (55), 148 (100), 122 (84); $[\alpha]_D$, +52.8°; UV λ_{max} (MeOH) nm: 377 (ε800), 350 (1700), 326 (1700), 285 (1100), 264 (144000), 251 (103000), 238 (49000), 226 (19000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2100 and 2200 (–C≡C–), 860 (epoxide); ^1H NMR: δ2.00 (3H, *s*), 2.96 (2H, *d*-like, J = 3 Hz), 3.43 (1H, *t*-like, J = 3 Hz).

Catalytic hydrogenation of **1**. (i) The epoxide **1** (5 mg) was hydrogenated (PtO_2 2 mg, EtOH 3 ml, 1 hr) and the product purified by silica gel CC with CH_2Cl_2–MeOH (49:1) yielding 1-tridecanol; EIMS, *m/z* (rel. int.): 182 (2, $[M-H_2O]^+$), 154 (4), 125 (6), 111 (13), 97 (32), 83 (50), 69 (65), 55 (83), 43 (100); ^1H NMR: δ0.88 (3H, *t*, J = 7 Hz), 1.2 (22H, *br s*), 3.64 (2H, *t*, J = 6 Hz). (ii) The epoxide **1** (5 mg) was hydrogenated (5% Pd on $BaSO_4$ 9 mg, *n*-hexane, 1 hr) and the products sepd by silica gel CC with CH_2Cl_2–CCl_4 (1:2) to give a satd epoxide **3b** (1 mg).

$LiAlH_4$ *reduction of* 1,2-*epoxytridecanes* (**3a** and **3b**). (i) A mixture of (*R*)-(+)-1,2-epoxytridecane (**3a**) (1.0 g) [8] and $LiAlH_4$ (2.4 g) in Et_2O was refluxed for 16 hr. After usual work-up 0.91 g of (*S*)-(+)-2-tridecanol (**4a**) was obtained. (ii) The saturated epoxide **3b** (1 mg) from **1** was treated similarly with $LiAlH_4$ to give an enantiomeric alcohol **4b** (0.7 mg).

Preparation of MTPA esters. (i) (*S*)-(+)-2-tridecanol (**4a**, 100 mg) was converted to the MTPA ester **5a** (158 mg) of (*R*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetic acid according to the method of ref. [14]. ^1H NMR: δ3.57 (3H, –OMe, with *ca* 1 Hz long-range coupling). (ii) The tridecanol (**4b**) from **1** was converted similarly to a MTPA ester **5b** (1.2 mg). ^1H NMR: δ3.55 (3H, –OMe, with *ca* 1 Hz long-range coupling). (iii) Racemic 2-tridecanol **4c** (126 mg) was converted similarly to the corresponding diasteromeric mixture of MTPA esters **5c** (155 mg). ^1H NMR: δ3.55 and 3.57 (–OMe).

HPLC analysis of MTPA esters, **5a**, **5b**, *and* **5c**. HPLC [μ-Porasil, *n*-hexane–CH_2Cl_2 (4:1), 1 ml/min] of **5a**, **5b**, **5c** showed R_ts of 14.6, 16.2, 14.6 and 16.2 min, respectively.

1-*Tridecen*-3,5,7,9,11-*pentayne* (**2**). Highly unstable yellow crystals, decomposing rapidly; high resolution MS, *m/z* 162.0454 $[M]^+$ (calc. for $C_{13}H_6$: 162.0470); EIMS, *m/z* (rel. int.): 162 (100), 136 (32), 110 (29); UV λ_{max} (MeOH) nm 410 (ε1500), 378 (5500), 352 (5800), 330 (3100), 308 (1500), 285 (58300), 269 (63500), 264 (72900), 257 (55700), 252 (sh, 46700), 245 (sh, 31100); IR ν_{max} ($CHCl_3$) cm^{-1}: 3020, 2225 and 2190 (–C≡C–), 390 (–CH=CH_2); ^1H NMR: δ2.00 (3H, *s*, Me) 5.7–6.0 (3H, *m*, –CH=CH_2).

HPLC analysis of **2** *in different tissues.* Each Me_2CO extract from respective tissue of single burdock root was submitted to HPLC (μ-Porasil, *n*-hexane) and peak areas corresponding to **2** (R_t 7.5 min) were compared. Relative amounts of **2** from peeled, peeled and $CuSO_4$-treated root tissues, and root epidermal tissue were in the ratio of 1, 30, and 4, respectively.

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