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2-HYDROXY-7-METHOXYCADALENE. THE PRECURSOR OF LACINILENE C 7-METHYL ETHER IN *GOSSYPIUM*

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Key Word Index—*Gossypium;* Malvaceae; cotton; sesquiterpenoid; byssinosis; (+)-lacinilene C 7-methyl ether; (+)-lacinilene C; cotton dust; biosynthesis; 2,7-dihydroxycadalene; 2-hydroxy-7-methoxycadalene.

Abstract—Two sesquiterpenoid naphthols, 2,7-dihydroxycadalene and 2-hydroxy-7-methoxycadalene, have been isolated from green and field-dried cotton bracts. These naphthols rapidly autoxidize on silica gel to lacinilene C and lacinilene C 7-methyl ether, respectively. The latter compound has been implicated as a causative of byssinosis. Lacinilene C and its methyl ether derivative isolated from field-dried cotton leaves and bracts were optically active, indicating that the lacinilenes are produced enzymatically from the naphthols. Therefore, bioassays for byssinotic activity using racemic synthetic lacinilene C 7-methyl ether, rather than the naturally occurring optically active compound, must be scrutinized carefully.

INTRODUCTION

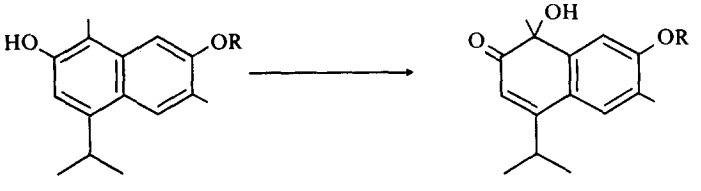
The plant trash present in mechanically harvested raw cotton is predominately bract and leaf parts trapped in the lint. During fiber processing these fragments micronize and produce a dust, which upon inhalation by some mill workers causes chronic respiratory distress. The symptoms of this distress are referred to as byssinosis. We have isolated and identified two yellow fluorescent sesquiterpenoids, lacinilene C (**3**), and lacinilene C 7-methyl ether (**4**) from field dried cotton bracts and dust ([1–2]; G. A. Greenblatt, unpublished results). The latter compound has been implicated as a causative of byssinosis [3]. The isolation of **4** was subsequently reported by Jeffs and Lynn [4], and its structure has been confirmed by synthesis [5, 6].

In this paper we report the isolation and identification of two aromatic sesquiterpenoid naphthols (**1** and **2**) that appear to be the biosynthetic precursors of compounds **3** and **4**.

RESULTS AND DISCUSSION

The sesquiterpenoid naphthols (**1** and **2**) and the lacinilenes (**3** and **4**) were observed together in aqueous methanol extracts of green and field-dried leaves and bracts of several commercial cotton cultivars. The naphthols occurred at the highest levels in mature green bracts. The cultivars 'Deltapine 55', 'Acala SJ-2' and 'Stoneville 213' appeared to contain higher concentrations than 'TAMCOT SP 37' or 'TAMCOT CAMD-E'. When applied to silica gel TLC plates and exposed to air overnight or to 100° heat for 10 min, the sesquiterpenoid naphthols (**1** and **2**) were converted to yellow fluorescent compounds that had identical R_f values in Et_2O–hexane (1:1) to those of lacinilene C (**3**) and lacinilene C 7-methyl ether (**4**), respectively. The ^1H NMR and MS of the conversion products also were the same as those of compounds **3** and **4**. Thus, the sesquiterpenoid naphthols are autoxidative precursors of the lacinilenes (**3** and **4**), and most likely have the same basic carbon skeleton.

The high resolution mass measurement of naphthol **2** confirmed its formula as $C_{16}H_{20}O_2$. Its ^1H NMR spectrum (see Experimental) showed three aromatic proton singlets, and aromatic methoxy, isopropyl, and two methyl groups. A large bathochromic shift (18 nm) in the UV spectrum after raising the pH of an EtOH solution



1 R = H
2 R = Me

3 R = H
4 R = Me

indicated that the remaining oxygen function was in a phenolic hydroxyl. These data, and its conversion to lacinilene C 7-methyl ether (**4**) indicated that the compound was 2-hydroxy-7-methoxy-cadalene (**2**).

Compound **2** occurred as a by-product in the synthesis of lacinilene C 7-methyl ether (**4**) by McCormick *et al.* [6, 7], and as an intermediate in the Jeffs and Lynn [5, 8] synthesis of **4**. We obtained a mp of 169–171.5° (hexane–Et_2O) for **2**, which agrees with that of Shafer (mp 169.6–172.7°) [7]. Our ^1H NMR spectrum was also in excellent agreement with that obtained by Shafer [7].

The naphthdiol **1** was labile, and on concentrating the compound a red oil formed slowly. Due to its instability, the compound has not been fully characterized. However, its ^1H NMR and MS (see Experimental) were in agreement with structure **1**. These data and its rapid oxidation to lacinilene C (**3**) indicated that the compound was 2,7-dihydroxycadalene (**1**).

The 1-alkyl-2-naphthols have previously been shown to react with oxygen, and the resulting 1-alkyl-1-hydroperoxy-2-naphthalenones have been isolated and characterized [9]. As expected, these hydroperoxides are easily reduced to the hydronaphthalenones. Therefore, one possible mechanism for the formation of the lacinilenes is by autoxidation of the cadalenes **1** and **2**. This mechanism and the occurrence of a compound such as **2** was proposed by Lynn [8] to explain the presence of the lacinilenes in field-dried cotton bracts but not in green bracts. Although Jeffs and Lynn [5, 8] found no lacinilenes in green bract tissue, we found them in young and mature green bracts in six commercial cultivars. Only in two cultivars, 'TAMCOT SP-37' and 'TAMCOT CAMD-E', were the lacinilenes present at a level that made detection difficult. Furthermore, both healthy and *Verticillium* wilt-diseased tissue contained the lacinilenes.

Jeffs and Lynn [5, 8] reported that lacinilene C 7-methyl ether (**4**) was not optically active. However, we found that lacinilene C (**3**) and its methyl ether derivative (**4**) isolated from field-dried bracts were optically active. Lacinilene C (mp 65–74°, hexane–Et_2O) had $[\alpha]_D^{25} = +37.7°$ (EtOH, $c = 0.159$) and lacinilene C 7-methyl ether (mp 106–111°, hexane–Et_2O) had $[\alpha]_D^{25} = +109.6°$ (EtOH, $c = 0.183$).

The occurrence of the lacinilenes in an optically active form indicates that they are formed enzymatically *in situ*. In addition, they are formed non-enzymatically by autoxidation, perhaps in dried tissue and certainly during extraction and purification. The wide melting range of the lacinilenes is probably due to the presence of a mixture of optically active and racemic forms, since spectral data indicated pure samples. Our melting range for **4** is close to that reported by McCormick *et al.* (mp 102–104°) for the synthetic racemate [7], but differs considerably from the melting range reported by Lynn (mp 117–118°) [8] for their synthetic racemate.

Because lacinilene C 7-methyl ether (**4**) is present in an optically active form in field-dried bracts, bioassays for byssinotic activity using synthetic material must be interpreted with caution. Furthermore, since 2-hydroxy-7-methoxycadalene (**2**) is a naturally occurring compound that autoxidizes to lacinilene C 7-methyl ether (**4**), it must also be considered as a potential causative of byssinosis and should therefore be examined for biological activity.

EXPERIMENTAL

Extraction and isolation. Freeze dried green bracts from mature bolls of Acala SJ-2 were ground in a blender to a fine powder. Although the lacinilenes and the cadalenes could be extracted with H_2O, they were more efficiently extracted in 4 hr with 30% aq. MeOH (100 ml solvent/g powder). The slurry was filtered, NaCl (10 g) was added to the aq. MeOH, and this soln was extracted with hexane–EtOAc (17:3, 3 × 20 ml). After drying over dry Na_2SO_4, the organic soln was evapd to dryness. The residue was chromatographed on a Si gel column eluting with increasing concentrations of EtOAc in hexane. The elution order was compounds **2**, **4**, **1**, and **3**. Compounds **2**, **3**, and **4** were purified by TLC (Si Gel) in hexane–Et_2O (1:1). Compound **1** was purified by reverse phase HPLC eluting with MeOH–H_2O (3:2).

2-Hydroxy-7-methoxycadalene (**2**). MS: *m/e* (rel. int.): 244.1468 (calc. for $C_{16}H_{20}O_2$: 244.1463, 74), 230 (18), 229 (100), 213 (25). ^1H NMR [90 MHz, $(CD_3)_2CO$]: δ 1.33 (6H, *d*, $J = 6.8$ Hz), 2.34 (3H, *bs*, Ar-Me), 2.45 (3H, *s*, Ar-Me), 3.69 (1H, *septet*, $J = 6.8$ Hz), 3.98 (3H, *s*, OMe), 6.99 (1H, *s*, Ar-H), 7.18 (1H, *s*, Ar-H), 7.82 (1 H, *s*, Ar-H). UV λ_{max}^{EtOH} nm (ε): 335 (3,100), 320 (sh), 290 (4,900), 240 (57,600), 220 (sh); $\lambda_{max}^{EtOH/HCl}$ nm (ε): 334 (3,000), 291 (4,800), 240 (57,600), 220 (sh); $\lambda_{max}^{EtOH/NaOH}$ nm (ε): 352 (4,000), 315 (sh), 300 (4,700), 288 (4,500), 245 (42,800). IR ν_{max}^{KBr} cm^{-1}: 1605, 1575.

2,7-Dihydroxycadalene (**1**). MS: *m/e* (rel. int.): 230 (97), 216 (20), 215 (100), 201 (13), 200 (16), 186 (11), 147 (23). ^1H NMR [90 MHz, $(CD_3)_2CO$]: δ 1.32 (6H, *d*, $J = 6.3$ Hz) 2.35 (3H, *s*, Ar-Me), 2.35 (3H, *bs*, Ar-Me), 3.66 (1H, *septet*, $J = 6.3$ Hz), 6.92 (1H, *s*, Ar-H), 7.23 (1H, *s*, Ar-H), 7.80 (1H, *s*, Ar-H), 7.95 (1H, *s*, –OH, exchanged with D_2O), 8.43 (1H, *s*, –OH, exchanged with D_2O).

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