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A DITERPENE FROM *BIDENS PILOSA*

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Key Word Index—*Bidens pilosa*; Compositae; diterpene; phytyl heptanoate.

Abstract—A new diterpene, phytyl heptanoate, has been isolated from *Bidens pilosa*, and its structure confirmed by chemical synthesis.

INTRODUCTION

Bidens pilosa is found in waste places throughout the Philippines. It was investigated because of its wide range of medicinal applications. Its leaves are used as treatment for rheumatism, sore eyes, abdominal troubles, ulcers, swollen glands and toothaches, among others [1]. A number of earlier studies reported the isolation of sterols [2], monoterpenes [3], sesquiterpenes [3], flavones [4], flavonoids [5], glucosides [6] and hydrocarbons [2, 7]. We now report the isolation and identification of a novel diterpene (**1**) from the same species.

RESULTS AND DISCUSSION

The non-polar fractions of the chloroform extract of the leaves of *B. pilosa* afforded stigmasterol, squalene, a mixture of elaidic acid and behenic acid, and a novel diterpene, phytyl heptanoate. The structures of the first four compounds were identified by NMR, IR mass spectra and comparison with spectra obtained from authentic samples. The structure of phytyl heptanoate was elucidated by NMR, IR, mass spectrometry and, finally, synthesis of the compound from phytol and heptanoic acid using dicyclohexylcarbodiimide (DCC) as dehydrating agent and dimethylaminopyridine (DMAP) as catalyst [8].

The ^{1}H NMR spectrum of phytyl heptanoate revealed the presence of an olefinic proton at δ5.33 (*t*, J = 7.0 Hz) which was coupled to methylene protons at δ4.59 (*d*, J = 7.0 Hz). On chemical shift grounds, the methylene may be attached to an oxygenated carbon. Possibly the compound had an ester functionality (C=O stretch at 1724 cm^{-1} and C–O–C stretch at 1203 cm^{-1}). A methyl group attached to an olefinic carbon gave rise to a resonance at δ1.69. The triplet at δ2.01 suggested methylene attached to an olefin, while the triplet at δ2.29 and quintet at δ1.62 may be assigned to methylene groups attached α and β, respectively, to a carbonyl, which is supported by a strong correlation in COSY.

The mass spectrum of phytyl heptanoate revealed a molecular ion peak at *m/z* 408. The most characteristic peak was the base peak *m/z* 278 which could have been generated by a McLafferty rearrangement to a diene and an acid.

The acidic part of the molecule had a M_r of 130 and a survey of acids with this mass revealed the possibility of heptanoic acid, $C_7H_{14}O_2$ and a $C_6H_{12}O_3$ compound. The latter was ruled out as the NMR and IR spectra do not support the presence of alcohol or ether in the acid. The alcohol portion of the compound has a molecular formula, $C_{20}H_{39}OH$. Among the list of alcohols with this formula, which is consistent with the ^{1}H NMR spectral data, is phytol, a diterpene alcohol. Therefore, the peak at *m/z* 278 may be as shown overleaf.

Based on the previously mentioned analysis it can be inferred that the compound is phytyl heptanoate, and to confirm this structure, synthesis work was carried out. Phytyl heptanoate was prepared by esterification of heptanoic acid and phytol catalysed by sulphuric acid which afforded a 2.86% yield. To obtain a higher yield, esterification was again carried out using DCC as dehydrating agent and DMAP as catalyst which afforded a 64.8% yield. Comparison of the ^{1}H NMR, IR and mass spectra of the synthesized phytyl heptanoate and the isolated compound revealed that the two were identical.

EXPERIMENTAL

Isolation. A series of chromatographic separations was conducted on the first fr. collected with the following solvent systems as eluent: hexane–DCM (5:1, 10:1, 2:1). Final separation was accomplished by prep. TLC using hexane–DCM (2:1) as developing solvent. Phytyl heptanoate (4.0 mg), an oil, was obtained. The third fr. obtained from the first column was rechromatographed

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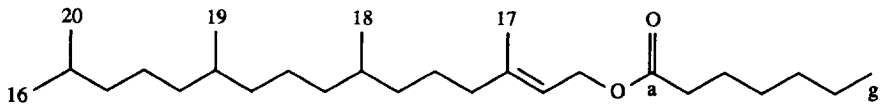

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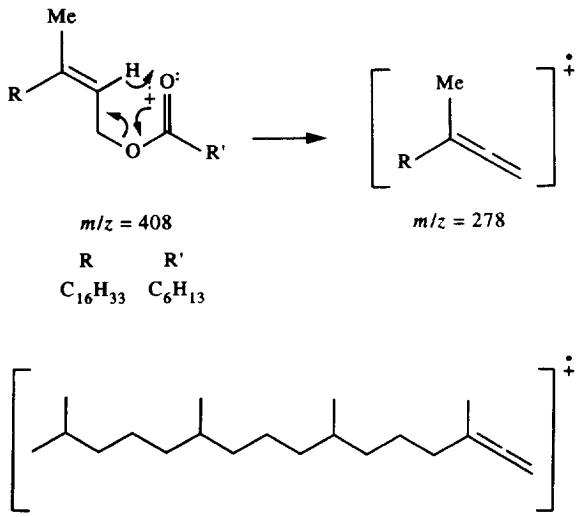

using $CHCl_3$–Et_2O (4:1) as eluent. Prep. TLC was carried out on the third fr. collected to afford stigmasterol (20.5 mg) which was recrystallized in hexane. The third fr. from the first column was rechromatographed twice using $CHCl_3$–Et_2O (4:1) as eluent. Final sepn was accomplished by TLC, using the same developing solvent to afford a mixture of behenic acid and elaidic acid (4.0 mg). The least polar fr. from the first column was rechromatographed ×3 using hexane–DCM (10:1) as eluent to afford squalene (7.40 mg).

Phytyl heptanoate (**1**). Oil, bp 159°, $[\alpha]_D^{28}$ −0.89° (EtOH) MS *m/z* 408. IR ν_{max} cm^{-1}: 1724 (C=O), 1203 (C–O–C), 1522 (C=C), 3015 (=CH), 2860 and 2927 (C–H), 1466 and 1379 (C–H). ^1H NMR (500 MHz, $CDCl_3$): δ4.59 (2H, *d*, *J* = 7 Hz, H-1), 5.33 (1H, *t*, *J* = 7 Hz, H-2), 2.01 (2H, *t*, H-4), 1.53 (1H, *m*, H-15), 0.866 (6H, *d*, *J* = 6.40 Hz, H-16 and H-20), 1.69 (3H, *s*, H-17), 0.85 (6H, *d*, *J* = 6.4 Hz, H-18 and H-19), 2.29 (2H, *t*, H_b), 1.62 (2H, *m*, H_c), 0.88 (3H, *t*, H_g), 1.20–1.35 (26H, *m*, CH_2).

Synthesis. (a) A soln of heptanoic acid (3.4 mmol), phytol (3.7 mmol), benzene and 2 drops of conc. H_2SO_4 was refluxed for 4 hr. The soln was washed with 0.5 M HCl, followed by satd Na_2CO_3, then dried over Na_2SO_4 and the solvent evapd *in vacuo* to afford phytyl heptanoate (2.86% yield) which was purified by CC.

(b) A soln of heptanoic acid (10.13 mmol) and phytol (10.81 mmol), DCC (1.3 mmol), DCM and DMAP (1 mmol) was refluxed until esterification was complete. Precipitated urea was filtered off and the filtrate evaporated *in vacuo*. The residue was taken up in DCM and washed with 0.5 M HCl, followed by satd $NaHCO_3$ soln and dried over Na_2SO_4. The solvent was evapd *in vacuo* to afford phytyl heptanoate (64.8% yield) which was purified by gravity column using DCM–hexane (2:1) as eluent.

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