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THYMOL DERIVATIVES FROM *EUPATORIUM GLECHONOPHYLLUM**

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Key Word Index—*Eupatorium glechonophyllum*; Compositae; thallus; chromene derivatives; thymol derivatives.

Abstract—The known 10-acetoxy-8,9-epoxythymolisobutyrate and the new 8,9-dihydroxy-10-acetoxythymolisobutyrate and 8,9,10-trihydroxythymol have been isolated from the thallus of *Eupatorium glechonophyllum*. The acetonide of the last compound was also obtained. The possibility that the new compounds are artefacts is discussed.

INTRODUCTION

Triterpenes, steroids, aromatic acids and a number of *p*-hydroxyacetophenone derivatives, mainly condensed with a 2,2-dimethyl-2H-pyran ring, have been isolated from the leaves of *Eupatorium glechonophyllum* Less [1, 2]. The structure of some thymol derivatives, which had been obtained in semimicro amounts, were not determined [2].

A thallus extract of the plant afforded satisfactory amounts of four thymol derivatives, whose structures were established as **1**–**4**. Compound **1** has been reported in Compositae [3], whereas **2** and **3** are new; compound **4** is considered to be an artefact of **3**.

RESULTS AND DISCUSSION

The four thymol derivatives gave a violet colour with sulphuric acid on TLC, similar UV and IR spectra and the signals of a 1,2,4-trisubstituted aromatic ring in the ^{1}H NMR spectra (see Experimental). The strong absorptions of unconjugated carbonyls (ν_{max} 1730 cm^{-1}) in the IR spectra of compounds **1**, $C_{16}H_{20}O_5$, and **2**, $C_{16}H_{22}O_6$, were assigned to an acetoxyl (ν_{max} 1235 cm^{-1}) and to an isobutyryl moiety, after examination of proton resonances.

The structures **1** and **2** follow from the remaining ^{1}H NMR signals. The former is coincident with the thymol epoxide reported from *Wedelia forsteriana* Endl [3], while the latter is the corresponding diol.

On consideration of the mass spectra reported in the literature, e.g. refs [4, 5], the mass fragmentation of these thymol diesters can be rationalized in the following terms. The C-10 substituent is lost through two different pathways: (i) in the form of the corresponding acid [4, 5]; (ii) by cleavage of the linkage between C-8 and C-10, to give most likely an oxonium ion [5]. Conversely the phenolic substituent is lost in the form of a ketene derivative. The priority of the losses is determined by the nature of the substituents. Nevertheless the relative position of the two ester groups can be determined. Following these rules the mass fragmentations of **1** and **2** are in agreement with the proposed structures.

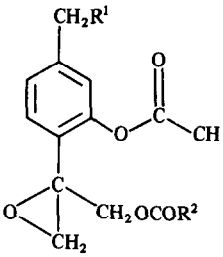
The loss of acetic acid from the molecular ion needs further comment. A six membered transition state was invoked to explain the loss of cinnamic acid from tinifoline, **1a** [5] and the same mechanism should be operating for **1** and **2** (for the latter see Scheme 1). The concerted loss of dimethyl ketene and water, which occurs in the mass spectrum of **2**, can be rationalized through a six membered transition state (Scheme 1). The ion at *m/z* 162 which is formed in the mass fragmentation of both **1** and **2**, is coincident with the molecular ion of a compound isolated by Bohlmann from *Helenium* genus [6]. The fragmentation which follows is in fact similar, notably the loss of the hemiacetalic hydroxyl (see also ref. [5]).

The crystalline monomethyl derivative, $C_{11}H_{16}O_4$ (**3a**), of the phenol (UV λ_{max}^{NaOMe} 291 nm) **3**, $C_{10}H_{14}O_4$, gave a diacetyl derivative (**3b**), which still contained a free hydroxyl group (IR ν_{max} 3520 cm^{-1}) and a triacetyl derivative (**3c**), on the use of more drastic conditions. This behaviour and the spectral data supported the structure of a trihydroxythymol (**3**). Coincidently the mass fragmentation of **3a** showed a base peak, $[M - CH_2OH]^+$, from which the elements of water, methanol and ethanol were lost. In the last two losses the methoxy group must be involved through the so called '*ortho* effect' [7], as represented in Scheme 2. In the mass spectrum of **3** the same ions at *m/z* 149 and 135 were formed by the corresponding losses of water§ and methanol. These considerations were conclusive for the assignment of the structure **3**. Transformation of **2** into **3** in alkali solution established the relationship between the two compounds.

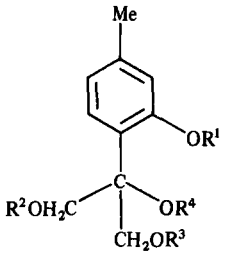
A second crystalline phenol (UV λ_{max}^{NaOMe} 291 nm), **4**, $C_{13}H_{18}O_4$, less polar than **3**, was isolated. In the ^{1}H NMR spectrum the usual signals of a thymol skeleton are joined with those of two different (singlet and quartet, respectively) methylene groups and two *gem*-dimethyl groups (see Experimental). Structure **4** was formulated and confirmed by the successive losses in the mass spectrum of 9-CH_2OH and ethyl acetate from the molecu-

*A preliminary communication of this work was presented at the 13th International Symposium on the Chemistry of Natural Products, Pretoria (1982).

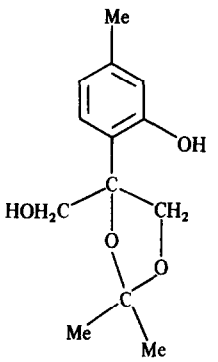
§This loss occurs very likely through two different pathways



	R^1	R^2
1	H	Me
1a	OH	CH=CH–Ph



	R^1	R^2	R^3	R^4
2	COCH(Me)Me	COMe	H	H
3	H	H	H	H
3a	Me	H	H	H
3b	Me	COMe	COMe	H
3c	Me	COMe	COMe	COMe



4

lar ion. Compound **4** was prepared from compound **3** (in acetone in the presence of H_2SO_4) and it was evidently formed from the latter during the separation.

The possibility that **2** was an artefact of **1** was also considered. The presence of **2** in old samples of **1** was noted, moreover **1** showed optical activity, whereas **2** had $[\alpha]_D = 0$. Partial or total transformation of **1** into **2** seemed to be very likely when a fresh extract gave **1** in major and **2** in minor yield. Conversion of **2** into **3** on long term storage did not take place even though the presence of two more polar products, which could not be investigated due to the lack of material, was demonstrated. These products were absent in the extracts and it was concluded that **3** is a natural product.

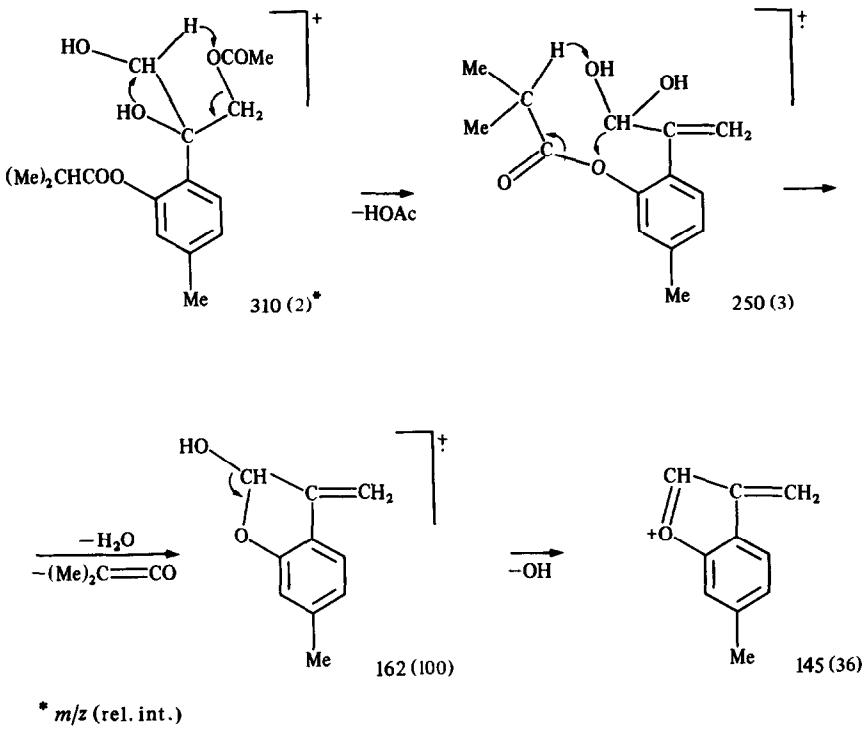
EXPERIMENTAL

General. Mps: uncorr; Elemental analyses: in agreement with molecular formulae. ^{1}H NMR: 60 MHz; MS: direct inlet, 70 eV.

Isolation. The EtOH extract (17.5 g) of thallus (0.5 kg) of *E glechonophyllum* [1] was partitioned between H_2O and $CHCl_3$ (residue 3.2 g), and then H_2O and EtOAc (1.6 g) Extended CC and prep. TLC of the $CHCl_3$ extract gave encecalin (250 mg) β-sitosterol (55 mg) and friedelanol (40 mg) as major products, desmethylencecalin, desmethoxyencecalin, methyl- and ethylencecalol, β-amyrin, β-amyrin acetate and friedelin as minor components and compound (**1**, 20 mg). The *p*-hydroxyacetophenone derivatives were identified by comparison with the samples previously isolated from the leaves of the plant [1, 2]. The EtOAc extract gave the thymol derivatives **2** (30 mg), **3** (50 mg) and **4** (55 mg), which were coincident with the previously unidentified components of the leaf extracts [2]. The EtOAc extract afforded also (*inter alia*) caffeic acid, identified by comparison with an authentic specimen.

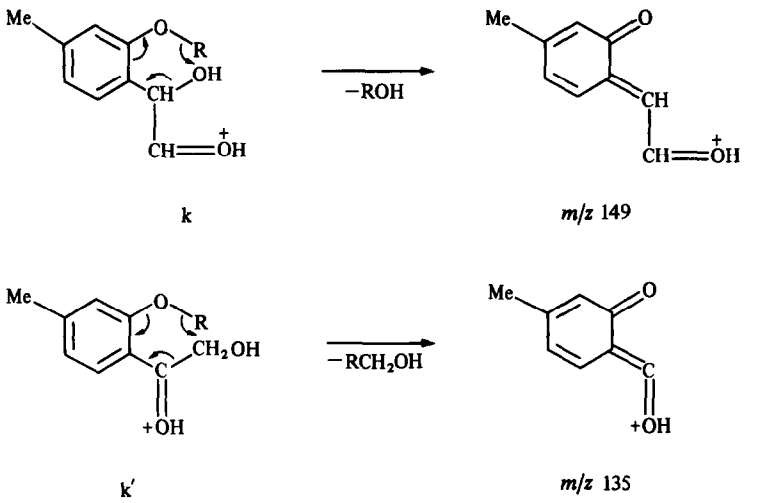
10-*Acetoxy*-8,9-*epoxythymolisobutyrate* (**1**). $C_{16}H_{20}O_5$, oil, $[\alpha]_D^{24} = +35$ (*c* 0.3, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε). 276 (3.42), 281 sh (3.40); ^{1}H NMR (CCl_4) and IR ($CHCl_3$) were in agreement with those reported in ref. [3]; MS *m/z* (rel. int.): 292 $[M]^+$ (1), 232 $[M-AcOH]^+$ (8) 219 $[M-CH_2OAc]^+$ (7), 162 $[232-Me_2C{=}CO]^+$ (60), 149 $[219-Me_2C{=}CO]^+$ (55), 145 $[162-OH]^+$ (33), 71 (58), 43 (100).

8,9-*Dihydroxy*-10-*acetoxythymolisobutyrate* (**2**) $C_{16}H_{22}O_6$, vitreous solid; UV λ_{max}^{MeOH} nm (log ε) 276 (3.58), 281 sh (3 56), IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3565, 3345, 1738–1730, 1630–1620, 1578, 1235; ^{1}H NMR (CCl_4): δ 6.8 (1H, *d*, J = 8 Hz, H-6), 6.54 (1H, *s* (*br*), H-2), 6 48 (1H, *d* (*br*), J = 8 Hz, H-5), 4.36 (4H, *s*, 2 × CH_2), 2.65–2.25 (1H, *m*, CH), 2.24 (3H, *s*, 7-Me), 2.0 (3H, *s*, COMe), 1 18 (6H, *d*, J = 6.5 Hz, 2 × Me); MS *m/z* (rel int.): 310 $[M]^+$ (2), 292 $[M-H_2O]^+$ (1), 250 $[M-AcOH]^+$ (3), 248 (3), 237 $[M-CH_2OAc]^+$ (7), 234 (4), 233 (2), 219 (8), 218 (8), 217 (36), 209



* *m/z* (rel. int.)

Scheme 1. Proposed fragmentation of compound **2**.



*k and k′ are two possible formulations of the ion (R = H, *m/z* 167, R = Me, *m/z* 181).

Scheme 2. Fragmentation of the ion $[M-CH_2OH]^+$ in the mass spectra of compounds **3** (R=H) and **3a** (R=Me) through the '*ortho* effect'.

(12), 203 (30), 187 (25), 182 (19), 181 (12), 178 (6), 167 [237 − Me_2 C=CO]$^+$ (36), 162 [250 − Me_2 C=CO − H_2O]$^+$ (100), 161 [162 − H]$^+$ (34), 149 [237 − Me_2 CH-COOH]$^+$ (66), 147 (24), 145 [162 − OH]$^+$ (36), 135 [161 − C_2H_2]$^+$ (54), 133 [162 − CHO]$^+$ (54), 121 (34), 119 (14).

Hydrolysis. Compound **2** (20 mg) was left for 2 hr at room temp. in 1 N KOH (MeOH, 2 ml). Prep. TLC of the reaction mixture afforded pure **3** (11 mg).

8,9,10-Trihydroxythymol (**3**). $C_{10}H_{14}O_4$, vitreous solid; UV λ_{max}^{MeOH} nm (log ε): 276 (3.34), 281 sh (3.32); λ_{max}^{MeONa}: 293 (3.62); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3520, 1630, 1575, 1512; ^{1}H NMR (CD_3COCD_3): δ 7.12 (1H, *d*, *J* = 8 Hz, H-5), 6.57 (1H, *d*, *J* = 2

Hz, H-2), 6.55 (1H, *dd*, J = 8 and 2 Hz, H-6), 4.44 (3H, *br*, exch. D_2O, 3 × OH), 3.87 (4H, *s*, 2 × CH_2), 2.19 (3H, *s*, 7-Me); MS *m/z* (rel. int.): 198 $[M]^+$ (13), 180 $[M-H_2O]^+$ (5), 167 $[M-CH_2OH]^+$ (95), 162 (37), 149 $[167-H_2O]^+$ (100), 135 $[167-MeOH]^+$ (71), 133 (28), 121 $[149-CO]^+$ (100), 109 (19), 107 (22), 105 (44), 93 $[121-CO]^+$ (27), 91 (47), 31 (87); m*. 140.9 (198 → 167), 132.9 (167 → 149), 98.3 (149 → 121), 71.5 (121 → 93).

Methyl ether (**3a**) Prepared from **3** with CH_2N_2. $C_{11}H_{16}O_4$, mp 93–94° (Et_2O–hexane); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3585, 3430, 1613, 1575 ^1H NMR ($CDCl_3$): δ 7.51 (1H, *d*, J = 8 Hz, H-5), 6.88 (1H, *d* (*br*), J = 8 Hz, H-6), 6.82 (1H, *s* (*br*), H-2), 4.11 (2H, *d*, J = 11 Hz, H-9, H-10), 3.89 (2H, *d*, J = 11 Hz, H-9′, H-10′), 3.6–2.6 (3H, *br*, exch. D_2O, 3 × OH), 3.86 (3H, *s*, OMe), 2.36 (3H, *s*, 7-Me); MS *m/z* (rel. int.): 212 $[M]^+$ (1), 211 (1), 194 $[M-H_2O]^+$ (3), 181 $[M-CH_2OH]^+$ (100), 163 $[181-H_2O]^+$ (64), 149 $[181-MeOH]^+$ (13), 135 $[181-EtOH]^+$ and $[163-CO]^+$ (71), 133 (8), 123 (18), 107 (5), 105 $[135-OCH_2]^+$ (96), 103 (7), 91 (25), 43 (12), 31 (7), m* 146.8 (181 → 163), 111.8 (163 → 135), 100.7 (181 → 135).

9,10-*Diacetoxymethylether* (**3b**). Prepared from **3** with pyridine–Ac_2O overnight $C_{13}H_{20}O_6$, oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1738–1730, 1613, 1580, 1240; ^1H NMR ($CDCl_3$): δ 7.28 (1H, *d*, J = 8 Hz, H-5), 6.73 (1H, *d* (*br*), J = 8 Hz, H-6), 6.67 (1H, *s* (*br*), H-2), 4.49 (4H, *s*, 2 × CH_2), 3.81 (3H, *s*, OMe), 2.36 (3H, *s*, 7-Me), 1.98 (6H, *s*, 2 × OCOMe)

8,9,10-*Triacetoxymethylether* (**3c**) Prepared from **3** with pyridine–Ac_2O (2 hr at reflux) $C_{15}H_{22}O_7$, oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1738, 1614, 1580, 1238; ^1H NMR ($CDCl_3$). δ 7.02 (1H, *d*, J = 8 Hz, H-5), 6.67 (1H, *d* (*br*), J = 8 Hz, H-6), 6.60 (1H, *s* (*br*), H-2), 4.70 (4H, *s* (*br*), 2 × CH_2), 3.74 (3H, *s*, OMe), 2.28 (3H, *s* (*br*), 7-Me), 2.08 (3H, *s*, 8-OCOMe), 1.94 (6H, *s*, 9- and 10-OCOMe).

9-*Hydroxy*-8,10-*isopropylendioxythymol* (**4**) $C_{13}H_{18}O_4$, mp 167–168° (Et_2O), $[\alpha]_D^{25}$ = 0; UV λ_{max}^{MeOH} nm (log ε): 275 (3.44) 281 sh (3.42); λ_{max}^{MeONa}. 291 (3.85); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3370, 1630, 1575; ^1H NMR ($CDCl_3$–CD_3OD, 4:1): δ 6.96 (1H, *d*, J = 8 Hz, H-5), 6.71 (1H, *d*, J = 2 Hz, H-2), 6.69 (1H, *dd*, J = 8 and 2 Hz, H-6), 4.50 (1H, *d*, J = 9 Hz, H-10), 4.24 (1H, *d*, J = 9 Hz, H-10′), 3.78 (2H, *s*, H-9 + H-9′), 2.29 (3H, *s*, 7-Me), 2.20 (1H, *s* (*br*), exchg. D_2O, 9-OH), 1.58 + 1.38 (3H + 3H, *s* + *s*, 2 × Me); MS *m/z* (rel. int.): 238 $[M]^+$ (5), 223 $[M-Me]^+$ (4), 207 $[M-CH_2OH]^+$ (91), 189 $[207-H_2O]^+$ (5), 163 $[M-Me_2CO-OH]^+$ (18), 161 $[189-CO]^+$ (15), 149 $[207-Me_2CO]^+$ (100), 145 $[163-H_2O]^+$ (11), 135 $[163-CO]^+$ (16), 133 (16), 121 $[149-CO]^+$ (100), 93 (18), 91 (20), 43 (28); m*. 172.6 (207 → 189); 8,9,10-trihydroxythymol (**3**, 20 mg) in Me_2CO (5 ml) with two drops of conc H_2SO_4 gave the acetonide 9-hydroxy-8,10-isopropylendioxythymol, coincident with compound **4**

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