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10-ACETOXY-9-CHLORO-8,9-DEHYDROTHYMOL AND FURTHER THYMOL DERIVATIVES FROM *ARNICA SACHALINENSIS*

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Key Word Index—*Arnica sachalinensis*; Asteraceae; flavers; thymol derivatives; thymol epoxides; chlorinated thymol derivative.

Abstract—The new thymol derivatives, 10-acetoxy-9-chloro-8,9-dehydrothymol, 10-acetoxy-8,9-dehydrothymol, 10-isobutyryloxy-8,9-dehydrothymol and 9-acetoxy-thymol, were isolated from flowerheads of *Arnica sachalinensis* and identified by their mass and NMR spectra. Furthermore, six new and five known thymol derivatives were identified as minor compounds mainly by TLC and GC-mass spectrometric analysis.

INTRODUCTION

Thymol derivatives are widely distributed in the Asteraceae, particularly within the tribes Helenieae, Heliantheae, Eupatorieae and Inuleae [1]. They have been shown to be common constituents of *Arnica* species [2]. Thymol, thymol methyl ether, thymol isobutyrate, 10-acetoxy-8,9-expoxythymol isobutyrate and 10-acetoxy-8-hydroxy-9-isobutyryloxy-thymol have been identified in flowerheads of the north east Asian endemic *A. sachalinensis* [3]. In contrast to other *Arnica* species *A. sachalinensis* lacks sesquiterpene lactones [3], well known for their ability to cause allergenic contact dermatitis [4–6]. Despite this, an allergic contact dermatitis has been described and the sensitizing agent was identified as 10-acetoxy-8,9-epoxythymol isobutyrate [7]. As some further fractions were positive in patch testing, we continued our investigations on thymol derivatives of *A. sachalinensis*. We now report the isolation and identification of some new thymol derivatives from the flowers of this species.

RESULTS AND DISCUSSION

Column chromatography (CC) of the dichloromethane extract of the flowerheads *of A. sachalinensis* afforded two fractions rich in thymol derivatives **1–11** and **12–15**, respectively. These fractions were separated by repeated CC on silica gel, followed by preparative TLC.

Compounds **1–3**, **5**, **7** and **11** gave dark orange colours after treatment with FBS B, indicating the presence of free phenolic hydroxyl groups. Treatment with 4-(4-nitrobenzyl)-pyridine reagent [8] indicated that compounds **8–15** are epoxides by an intensive blue colour of their reaction products.

The structure of the phenolic compound **1** clearly followed from its mass, ^{1}H and ^{13}C NMR spectra. The mass spectrum showed fragment ions at *m/z* 105, 91, 77, 65 and 51, generally found in the spectra of thymol derivatives [9, 10]. The ions at *m/z* 240 and 242, respectively, with an intensity proportion according to the natural distribution of the two chlorine isotopes indicated **1** as a chlorinated compound, which was further evident from the ions at *m/z* 205 $[M\text{-}Cl]^+$ and 204 $[M\text{-}HCl]^+$. Fragment ions at *m/z* 180 $[M\text{-}HAc]^+$, 163 $[M\text{-}CH_3COOCH_2]^+$, 162 $[204\text{-}CH_2CO]^+$, 161 $[204\text{-}CH_3CO]^+$, 73 $[CH_3COOCH_2]^+$ and 43 $[CH_3CO]^+$ indicated an aliphatic-bonded acetyl ester group.

The ^{1}H NMR spectrum (Table 1) showed the signals for a 1,3,4-trisubstituted benzene ring, two singlets for the methyl group attached to C-1 (H-7, δ 2.29 ppm) and for an acetyl group at δ 2.02. The spin system of the chemical equivalent protons at C-10 was recorded as doublet at δ 5.06, resulting from a weak coupling with a vinylic proton found at δ 6.34 (*t*), which therefore has to be assigned to H-9 ($J_{9,10}$ = 0.8 Hz), attached to the chlorine-substituted carbon. When the signal of H-5 at δ 6.95 was irradiated, a NOE was recorded for the signal of H-9, indicating a *trans*-configuration of the double bond.

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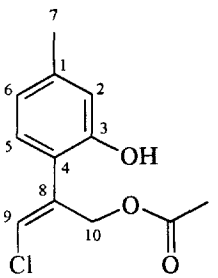

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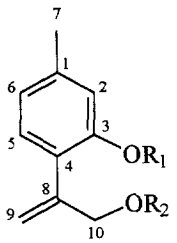
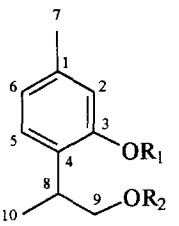
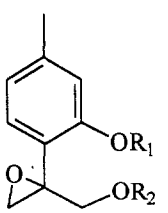


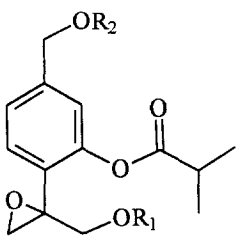
	R^1	R^2
2	H	Ac
3	H	*i*-But
4	*i*-But	Ac



	R^1	R^2
5	H	Ac
6	*i*-But	Ac
7	H	H



	R^1	R^2
8	*i*-But	Ac
9	*i*-But	*i*-But
10	*i*-But	C_4H_9CO
11	H	H



	R^1	R^2
12	Ac	*i*-But
13*	Ac	C_4H_9CO
14*	*i*-But	H
15*	C_4H_9CO	H

R^1, R^2 interchangeable

The structure of **1** as 10-acetoxy-9-chloro-8,9-dehydrothymol was additionally confirmed by its ^{13}C NMR spectrum (see Experimental), including gated-decoupling experiments, and its 2D-heteronuclear correlated $^{13}C/^1H$-HETCOR spectrum, which showed crosspeaks for seven hydrogen containing carbons, which could easily be assigned by their corresponding proton NMR signals. The remaining carbon signals in the spectra were due to five quaternary carbons. In the shift range of δ 121, were three signals found relatively close to each other (C-9, C-6 and C-4). The signals at δ 121.6 and 121.3 were assigned to C-9 and C-6, respectively, by their $^{13}C/^1H$ couplings in the 2D-HETCOR spectrum. The signal at δ 121 were assigned to C-4, previously found in this shift range for other thymol derivatives [3].

The possibility that this new chlorinated natural compound could be an artifact, built up by reaction with traces of HCl in dichloromethane during the extraction process, could be ruled out by identification of the compound in dried and powdered flowers, extracted with acetone-water (1:1) at room temperature, using TLC and GC-mass spectrometric analysis.

The fragmentation pattern in the mass spectra of

Table 1. ^{1}H NMR data of compounds **1-3** (300 MHz, $CDCl_3$) and **5** (200 MHz, $CDCl_3$)

proton	1	2	3	5
2	6.69 *d*	6.75 *s* (*br*)	6.74 *s* (*br*)	6.67 *d*
5	6.95 *d*	6.98 *d*	6.97 *d*	7.04 *d*
6	6.70 *dd*	6.70 *dd*	6.68 *dd*	6.72 *dd*
7	2.29 *s*	2.30 *s*	2.30 *s*	2.18 *s*
8				3.36 *m*
9	6.34 *t*	5.28 *dt*	5.26 *dt*	3.98 *dd*
		5.48 *dt*	5.44 *dt*	4.28 *dd*
10	5.06 *d*	4.75 *t*	4.75 *t*	1.34 *d*
OH		6.58 *s* (*br*)	6.84 *s* (*br.*)	5.84 *s* (*br*)
CH_3CO	2.02 *s*	2.14 *s*		2.08 *s*
$(CH_3)_2CHCO$			1.19 *d*	
			2.63 *m*	

J(Hz): **1**: 2,6=0.7; 5,6=8.4; 9,10=0.8; **2**: 2,6=0.8; 5,6=7; 9a,9b=1.4; 9a,10=9b,10=1.4; **3:** 2,6=1; 5,6=7.7; 9a,9b=1.3; 9a,10=9b,10=1.5; CH,CH_3 of i-But=7; **5:** 2,6<1; 5,6=7.7; 8,10=6.9; 8,9a=5.5; 8,9b=7.7; 9a,9b=10.9.

the isolated thymol derivatives **2** [M^+] *m/z* 206 and **3** [M^+] *m/z* 234 were very similar. Both compounds showed the base ion at *m/z* 146 ([M-60]$^+$ and [M-88]$^+$, respectively) and a fragment at *m/z* 133 ([M-CH_3CO-OCH_2]$^+$ and [M-C_3H_7CO-OCH_2]$^+$, respectively), indicating the presence of an aliphatic hydroxy function esterified with acetic acid (**2**) and with a saturated C_4-acid (**3**), respectively.

Their ^{1}H NMR spectra (Table 1) were similar to that of **1**, only differing by the presence of an AB-system for two olefinic protons at C-9 found at δ 5.48 and 5.28 for **2** and δ 5.44 and 5.26 for **3**, respectively. These signals showed an additional allylic coupling with a methylene group (H-10a/b) bearing an acetoxy and isobutyryloxy substituent, respectively (Table 1). Both thymol derivatives are new natural compounds, to the best of our knowledge.

The isolated compound **4** was identified by its mass and ^{1}H NMR data, as well as its chromatographic behaviour, as 10-acetoxy-8,9-dehydrothymol isobutyrate, previously reported from *Schkuhria multiflora* [11]. Mass and NMR spectral data showed the new compound **5** ([M]$^+$ *m/z* 208) to be the dihydroderivative of **2**. In contrast to the ^{1}H NMR spectrum of **2**, no olefinic H-9 was found. Instead of this the spectrum displayed the signals for one additional proton at δ 3.36 (H-8, *m*) and a doublet for a methyl group (H-10). Moreover, the signals for the methylene group bearing the acetoxy substituent appeared as AB part of an ABX-system (H-10 and H-8) at δ 4.28 (*dd*) and 3.98 (*dd*), respectively.

The presence of traces of **6** and **7** was established by TLC and GC-mass spectrometric analysis. Compound **6** was shown to be the dihydroderivative of **4**, since its mass spectrum exhibited a Mr of 278 (276 in **4**) and a fragmentation pattern analogous to **4**. Therefore, **6** should be 10-acetoxy-thymol isobutyrate, which has not been described up to now. The mass spectrum of **7**, showing the molecular ion at *m/z* 166 and the base peak at *m/z* 135 ([M-CH_2OH]$^+$), fully agreed with that of 9-hydroxythymol, previously isolated from *Kaunia* species [12].

Compound **8** was identified by its mass and ^{1}H NMR spectra as 10-acetoxy-8,9-epoxythymol isobutyrate, which has already been described as constituent of *A. sachalinensis* [3, 7]. Compounds **9** and **10** were minor components and identified by their chromatographic behavior and GC-MS analysis in comparison with **8**. The mass spectra of **9** and **10** exhibited a [M]$^+$ at *m/z* 320 and 334, respectively, and showed a fragmentation pattern analogous to that of **8**. The only differences resulted from an isobutyryloxy and a methylbutyryloxy group, respectively, at C-10 instead of the acetoxy group in **8**. Compound **9** has already been reported as a constituent of *A. amplexicaulis* [13] and *A. acaulis* [14], as well as of *Helenium mexicanum* [15], which also contained a 10-(2-methyl)-butyryloxy derivative, possibly identical to **10**. The mass spectrum of **11** [M^+ 180] was in agreement with 10-hydroxy-8,9-epoxythymol.

Compound **12** was isolated and identified as 7-isobutyryloxy-10-acetoxy-8,9-epoxythymol isobutyrate by its mass and ^{1}H NMR spectra, recently reported from *Calea nelsonii* [16]. The ^{1}H NMR data were in full agreement with those published for this compound. It is the first 7-substituted thymol derivative found in *Arnica*. Three further thymol derivatives of this type (**13–15**) could be detected as minor components and characterized by their chromatographic behaviour and by GC-mass spectrometric analysis. On this basis, compound **13** obviously differs from **12** only by the presence of a saturated C_5-acid at C-7 or C-10 instead of the isobutyryl group, whereas **14** and **15** are the analogous diesters with a free hydroxyl

group at C-7 or C-10 and an isobutyryloxy (**14**) and C_5-acyloxy (**15**) group at these positions, which could only be verified by their NMR spectra.

EXPERIMENTAL

Plant material

Arnica sachalinensis (Regl.) A. Gray was grown from seeds, originating from the Botanical Garden of the Academy of Sciences, Moscow, Russia. Voucher specimens (No. 12ff) are deposited at the herbarium of the Institute of Pharmaceutical Biology, Heinrich-Heine-Universität Düsseldorf.

Extraction and isolation

Dried and powdered flower heads (1200 g) were extracted with CH_2Cl_2 (Soxhlet) and the extract separated by CC on Sephadex LH-20 (Pharmacia) with MeOH as eluent. Frs (30 ml) containing thymol derivatives were combined after TLC and GC-MS monitoring to 2 fr containing compounds **1**–**11** (fr. I: 30.3 g) and **12**–**15** (fr. II: 4.3 g). CC of fr. I on silica gel (70–230 mesh) with toluene/Et_2O (4:1) and further separation by CC on silica gel with CH_2Cl_2 and prep. TLC (silica gel 60 plates, Merck) with toluene-Et_2O (4:1) or *n*-pentane-Et_2O (8:17) gave 20 mg **1** (oil), 15 mg **2** (oil), 55 mg **3** (yellow oil), 17 mg **4** (yellow oil) 15 mg **5** (yellow oil), 20 mg **8** (oil) and frs containing **3**, **4** and **6** (50 mg), **9** and **10** (1.3 g) and **2**, **5**, **7** and **11** (261 mg) as minor components. CC of fr. II (500 mg) on silica gel with CH_2Cl_2 afforded 3 frs containing compounds **12** (50 mg), **12** and **13** (181 mg) and **14** and **15** (16 mg), respectively. Compound **12** was further purified by prep. TLC (toluene-Et_2O, 4:1) to give 6 mg pure substance.

TLC

Silica gel 60 F_{254} (Merck), toluene-Et_2O (4:1). All compounds gave red to violet colored reaction products after treatment with anisealdehyde-H_2SO_4 in MeOH [8]. An intensive blue colour after treatment with 4-(nitro-benzyl)-pyridine reagent [8] proved compounds **8**–**15** to be epoxides. Treatment with FBS B-reagent [8] gave intense orange products with compounds **1**–**3**, **5** and **7**, indicating a free phenolic OH, whereas phenol ester derivatives **4**, **6**, **8**–**15** only gave weak yellow colours. R_f: **1** 0.37; **2** 0.38; **3** 0.48; **4** 0.60; **5** 0.35; **6** 0.55; **7** 0.14, **8** 0.46; **9** 0.49; **10** 0.52; **11** 0.37; **12** 0.37; **13** 0.38, **14** 0.27, **15** 0.29.

GC

Column OV-01, 25 m × 0.25 mm i.d.; temp. programmed 150°–270° at 10° min^{-1}; carrier N_2 at 1.2 ml min^{-1}, inj./detector temp. 300°. R_t (min): **1** 4.1; **2** 2.5; **3** 3.7; **4** 4.7; **5** 3.3; **6** 5.0; **7** 4.0; **8** 5.2; **9** 8.5; **10** 7.7; **11** 3.8; **12** 9.9; **13** 10.6; **14** 9.1; **15** 9.7.

GC-MS

EI, 70 eV. The MS of all compounds showed the characteristic peaks for thymol at *m/z* 105, 91, 77, 65 and 51 [9].

NMR

300 MHz (^1H), 75 MHz (^{13}C), $CDCl_3$, int. standard TMS.

10-*acetoxy*-9-*chloro*-8,9-*dehydrothymol* (**1**). MS, *m/z* (rel. int.): 240 and 242 [M^+] (15 and 5, respectively), 205 [M-Cl]$^+$ (8); 204 [M-HCl]$^+$ (17), 180 and 182 [M-CH_3COOH]$^+$ (18 and 6, respectively); 162 [204-CH_2CO]$^+$ (32), 161 [204-CH_3CO]$^+$ (10), 145 [180-Cl]$^+$ (100), 131 [204-CH_3COOCH_2]$^+$ (13), 115 (36), 73 [CH_3COOCH_2]$^+$ (7), 43 [CH_3CO]$^+$ (71). ^{13}C NMR: δ 140.4 (C-1), 116.8 (C-2), 153.4 (C-3), 121.0 (C-4), 130.0 (C-5), 121.3 (C-6), 21.2 (C-7), 135.3 (C-8), 121.6 (C-9), 62.7 (C-10), 20.7 (CH_3CO) and 171.5 (CH_3CO). ^1H NMR: Table 1.

10-*acetoxy*-8,9-*dehydrothymol* (**2**). MS, *m/z* (rel. int.): 206 [M]$^+$ (21), 164 [M-CH_2CO]$^+$ (6), 146 [M-CH_3COOH]$^+$ (100), 135 (35), 133 [M-CH_3COOCH_2]$^+$ (4), 131 (43), 117 (25), 115 (22), 43 [CH_3CO]$^+$ (58). ^1H NMR: Table 1.

10-*isobutyryloxy*-8,9-*dehydrothymol* (**3**). MS, *m/z* (rel. int.): 234 [M]$^+$ (22), 164 [M-$C_2H_6C{=}C{=}O$]$^+$ (10), 146 [M-C_3H_7COOH]$^+$ (100), 135 (32), 133 [M-$C_3H_7COOCH_2$]$^+$ (5), 131 (38), 117 (22), 115 (20), 71 [C_3H_7CO]$^+$ (28), 43 [71-CO]$^+$ (46). ^1H NMR: Table 1.

9-*acetoxy-thymol* (**5**). MS, *m/z* (rel. int.): 208 [M]$^+$ (15), 166 [M-$CH_2{=}C{=}O$] (2), 148 [M-CH_3COOH]$^+$ (86), 135 [M-CH_3COOCH_2]$^+$ (100), 133 (77), 121 (15), 115 (30), 43 [CH_3CO]$^+$ (78). ^1H NMR: Table 1.

9-*acetoxy-thymol isobutyrate* (**6**). MS, *m/z* (rel. int.): 278 [M]$^+$ (24), 236 [M-$CH_2{=}C{=}O$]$^+$ (7), 218 [M-CH_3COOH]$^+$ (22), 208 [M-$C_2H_6C{=}C{=}O$]$^+$ (85), 207 [M-C_3H_7CO]$^+$ (8), 205 [M-CH_3COOCH_2]$^+$ (1), 165 [208-CH_3CO, and/or 236-C_3H_7CO]$^+$ (18), 149 (96), 148 [208-CH_3COOH]$^+$ (100), 73 [CH_3CO-OCH_2]$^+$ (1), 71 [C_3H_7CO]$^+$ (83), 43 [CH_3CO or 71-CO]$^+$ (99).

10-*methylbutyryloxy*-8,9-*epoxy-thymol isobutyrate* (**10**). MS, *m/z* (rel. int.): 334 [M]$^+$ (3), 264 [M-$C_2H_6C{=}C{=}O$]$^+$ (1), 232 [M-C_4H_9COOH]$^+$ (4), 219 [M-$C_4H_9COOCH_2$]$^+$ (11), 189 [M-($CH_2O + C_4H_9COOCH_2$)]$^-$ (1), 177 (8), 162 [M-($C_4H_9COOH + C_2H_6C{=}C{=}O$)]$^+$ (100), 149 (1), 85 [C_4H_9CO]$^+$ (18), 71 [C_3H_7CO]$^+$ (11), 57 [85-CO]$^+$ (46), 43 [71-CO]$^+$ (77).

10-*hydroxy*-8,9-*epoxy-thymol* (**11**). MS, *m/z* (rel. int.): 180 [M]$^+$ (18), 163 [M-OH]$^+$ (20), 162 [M-H_2O]$^+$ (18), 150 [M-CH_2O]$^+$ (11), 149 [M-CH_2OH]$^+$ (100), 145 (17), 119 [M-($CH_2OH + CH_2O$)]$^+$ (22).

Compound **13**. MS, *m/z* (rel. int.): 392 [M]$^+$ (1), 362 [M-CH_2O]$^+$ (1), 332 [M-CH_3COOH]$^+$ (2), 321 [M-C_3H_7CO]$^+$ (1), 319 [M-CH_3COOCH_2]$^+$ (3), 307 [M-C_4H_9CO]$^+$ (1), 292 [362-$C_2H_6C{=}C{=}O$]$^+$ (2), 277 [M-$C_4H_9COOCH_2$]$^+$ (4), 264 [M-($C_4H_9CO + CH_3CO$)]$^+$

249 [M-($C_2H_6C{=}C{=}O + CH_3COOCH_2$)]$^+$ (97), 220 [362-($C_2H_6C{=}C{=}O + C_4H_9COOH$)]$^+$ (16), 115 $[C_4H_9COOCH_2]^+$ (4), 85 $[C_4H_9CO]^+$ (48), 73 $[CH_3COOCH_2]^+$ (4), 71 $[C_3H_7CO]^+$ (100), 57 [85-CO]$^+$ (84), 43 [71-CO and $CH_3CO]^+$ (99).

Compound **14**. MS, *m/z* (rel. int.): 336 [M]$^+$ (1), 307 [M-CHO]$^+$ (1), 265 [M-$C_3H_7CO]^+$ (2), 249 [320-$C_3H_7CO]^+$ (4), 235 [M-$C_3H_7COOCH_2]^+$ (2), 149 (24), 101 $[C_3H_7COOCH_2]^+$ (1), 71 $[C_3H_7CO]^+$ (92), 43 [71-CO]$^+$ (100).

Compound **15**. MS, *m/z* (rel. int.): 350 [M]$^+$ (1), 333 [M-OH]$^+$ (1), 321 [M-CHO]$^+$ (1), 279 [M-$C_3H_7CO]^+$ (1), 263 [333-$C_2H_6C{=}C{=}O]^+$ (3), 235 [M-$C_4H_9COOCH_2]^+$ (1), 149 (11), 115 $[C_4H_9COOCH_2]^+$ (3), 85 $[C_4H_9CO]^+$ (13), 71 $[C_3H_7CO]^+$ (86), 57 [85-CO]$^+$ (28), 43 [71-CO]$^+$ (100).

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