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# STEROIDS AND TRITERPENOIDS OF *ANTRODIA CINNAMOMEA*—A FUNGUS PARASITIC ON *CINNAMOMUM MICRANTHUM*

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**Key Word Index**—*Antrodia cinnamomea*; Polyporaceae; steroids; zhankuic acids D, E; triterpenoids; 15α-acetyl-dehydrosulphurenic acid; dehydroeburicoic acid; dehydrosulphurenic acid.

**Abstract**—Two ergostane related steroids, zhankuic acids D and E together with three lanosta related triterpenes, 15α-acetyl-dehydrosulphurenic acid, dehydroeburicoic acid, dehydrosulphurenic acid were isolated from the fruit body of the fungus *Antrodia cinnamomea*. Their structures were determined by spectral analyses and comparison with known compounds.

## INTRODUCTION

*Antrodia cinnamomea* Chang & Chou, sp. nov. (Zhan-Ku, family Polyporaceae, Aphyllophorales) is a new and exclusive fungus parasitic on the inner wall of the endemic species *Cinnamomum micranthum* (Hayata) Hayata [1]. It has been used for the treatment of food, alcohol and drug intoxication, diarrhoea, abdominal pain, hypertension, skin itches and liver cancer in Chinese folk medicine [2]. Preliminary pharmacological studies revealed that the ethanolic extract of this fungus possessed significant anti-serotonin and anticholinergic activities. In addition, the crude extract of the fruit bodies showed cytotoxicity against P 388 murine leukaemia cells at 4 $\mu g\,ml^{-1}$. Chemical investigation revealed that *Antrodia cinnamomea* was rich in steroids and triterpene acids. The pharmacological activities and the chemical constituents of crude extract of Zhan-Ku are quite different from those reported for *Ganoderma lucidum* [3,4]. Recently, three new ergosteroids named antcins, A, B and C were isolated and structures elucidated [5]. Among them, antcin A was confirmed by X-ray crystallographic analysis. In a previous communication we have isolated and elucidated three new ergosteroids, zhankuic acids, A (**1**), B (**3**) and C (**4**) from the fruit body of *Antrodia cinnamomea* [6]. Herein, we wish to report the isolation and structural elucidation of five additional compounds, zhankuic acids D (**2**) and E (**5**), 15α-acetyl-dehydrosulphurenic acid (**6**) dehydroeburicoic acid (**7**) and dehydrosulphurenic acid (**8**) from this fungus.

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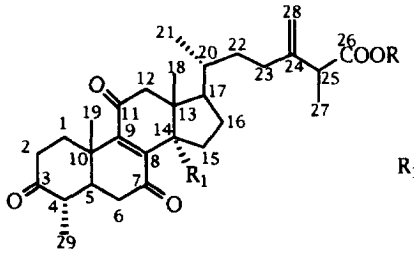
## RESULTS AND DISCUSSION

As reported in our previous paper, the ethyl acetate-soluble material of the ethanol extract of *Antrodia cinnamomea* was chromatographed on Sephadex LH-20 and silica gel columns. Four new compounds designated zhankuic acid D (**2**), zhankuic acid E (**5**), dehydrosulphurenic acid (**8**) and 15α-acetyl-dehydrosulphurenic acid (**6**) were isolated and characterized in addition to three previously isolated steroid acids, zhankuic acids, A (**1**), B (**3**) and C (**4**). One more known compound was identified as dehydroeburicoic acid (**7**) by spectral comparison with authentic sample.

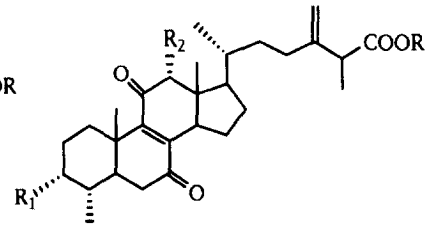
The molecular formulas of zhankuic acid D (**2**) ($C_{31}H_{45}O_5$) and E (**5**) ($C_{31}H_{47}O_6$) were established by $^{13}C$ NMR and DEPT as well as EI-mass spectra of **2** and **5**, respectively. The $^1H$ NMR spectra of Zhankuic acid D (**2**) and E (**5**) were very closely related to those of zhankuic acid A (**1**) and C (**4**), respectively. Studies of the mass fragmentation indicated that compound **2** was an analogue of **1** and compound **5** was an analogue of **4**. The molecular ion peaks at *m/z* 496 and *m/z* 514, respectively in the EI-mass spectra of **2** and **5** suggested that both compounds contained an ethyl moiety in the side chain. The $^1H$ NMR spectra of both **2** and **5** exhibited a quartet (2H, δ4.1, $J = 7.1$) and a triplet (3H, δ1.20, $J = 7.1$) indicating that **2** and **5** were 2,6-ethyl ester derivatives of **1** and **4**, respectively. These findings were supported by $^{13}C$ NMR spectra of **2** and **5**, which showed corresponding carbon signals of ester groups at δ14.2 (*q*), δ60.5 (*t*) and δ174.5 (*s*). Both **2** and **5** might be the artefacts from the esterification products of **1** and **4** in the procedure of fractionation.

Compounds **6**–**8** showed identical UV absorption pattern (250, 243, 237 nm) indicating that these compounds



**1** R= $R_1$= H

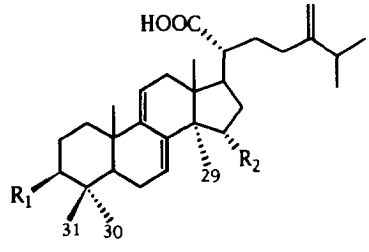
**2** R= $CH_2CH_3$ $R_1$= H



**3** R= H $R_1$= OH $R_2$= H

**4** R= H $R_1$= $R_2$= OH

**5** R= $CH_2CH_3$ $R_1$= $R_2$= OH



**6** $R_1$= OH $R_2$= OAc

**7** $R_1$= OH $R_2$= H

**8** $R_1$= $R_2$= OH

**9** $R_1$= OAc $R_2$= H

shared a common hetero-annular conjugated diene chromophore in the skeleton [7]. The molecular formulas of **6–8** ($C_{33}H_{50}O_5$, $C_{31}H_{48}O_3$, $C_{31}H_{48}O_4$) were established by high resolution EI-mass spectra, which showed molecular ions at *m/z* 526, 468 and 484, respectively. In addition, compounds **6–8** shared a common side chain fragment ($C_9H_{15}O_2$). This fragmentation pattern is quite similar to those reported for 24-methylene-containing triterpenoids [8, 9]. The $^1$H NMR, $^{13}$C NMR and DEPT spectra of **7** indicated the presence of four olefinic protons ($\delta$5.49, 5.32, 4.51, 4.59) and carbons ($\delta$121.3 *d*, 142.9 *s*, 146.7 *s*, 116.7 *d*), two secondary methyls ($\delta$0.96 *d*, 0.95 *d*), five tertiary methyls ($\delta$0.47 *s*, 0.83 *s*, 0.70 *s*, 0.73 *s*, 0.80 *s*), and an oxymethine proton ($\delta$3.04) and carbon ($\delta$78.1 *d*). It was suggested that the data of compound **7** was closely related to those reported for 24-methylenelanosta-7, 9(11)-diene-3$\beta$-ol [9]. However, the absence of a C-21 methyl doublet in **7** and the presence of a carboxylic acid carbon ($\delta$ 178.3) as well as formation of a monoacetate (**9**) suggested that compound **7** was dehydroeburicoic acid, which is a known compound reported in 1951 [10]. The assignment of $^1$H NMR data was completed by the application of a COSY experiment on compound **9**. The $^1$H and $^{13}$C NMR data are listed in the Experimental section. The $^1$H and $^{13}$C NMR spectra of **8** exhibited signals similar to those of compound **7** suggesting a close analogue. The only difference between **8** and **7** is that compound **8** contained two oxymethine carbons ($\delta$ 78.1 *d*, 73.8 *d*) and protons ($\delta$ 3.44, 4.80) instead of one in **7**. The location of the second hydroxyl was determined at C-15 due to the down-field shifts of C-14 and C-16. The configuration of the C-15 hydroxyl was determined as $\alpha$ due to the $\gamma$-effect of C-29 (upfield shift $\Delta\delta$ = − 7.7 compared with the corresponding signal in **7**). Thus, compound **8** was identified as dehydrosulphurenic acid, a new compound from natural source [11].

Compound **6** is a new analogue of compound **8**. The fragment ion peak at *m/z* 466 in the EI-mass spectrum of **6** indicated the presence of an acetoxyl group in **6**. The $^1$H and $^{13}$C NMR spectra of **6** were superimposable on those of **8**. The location of the acetoxy group was determined as the C-15 position due to the downfield shift

(+ 2.9 ppm) of C-15 and upfield shift (− 1.5 and − 3.8 ppm, respectively) of C-14 and C-16 when compared with those of compound **8**. Based on spectral evidence, compound **6** was established as 15α-acetoxy-24-methylene-lanosta-7,9(11)-dien-3β-ol-21-oic acid.

## EXPERIMENTAL

*General.* Mps uncorr. and were determined on a Fisher–Johns melting point apparatus. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV and IR spectra were taken with a Hitachi 150-120 and a JASCO A-100 IR spectrometers, respectively. The HREI-MS data were recorded on MAT 112S-JMS D300 and JEOL JMS-HX 110 spectrometers. $^1$H- and $^{13}$C NMR spectra were taken on a Bruker 300 AM spectrometer using TMS as internal standard.

*Plant material.* The fruit bodies of *Antrodia cinnamomea* were purchased from Ti-hua street in Taipei, 1987. A voucher specimen was preserved in the Institute of Marine Resources, National Sun Yat-sen University.

*Extraction and isolation.* The fourth fr. (3.3 g) obtained from LH-20 Sephadex column as reported previously [5] was chromatographed on a silica gel column with the solvent mixt. of MeOH and $CHCl_3$ (1%, 2%, 4%, and 8%) to give fr. *d* (53 mg). Purification of fr. *d* by prep. TLC provided 15-acetyl dehydroburicoic acid (**6**, 13 mg). The third fr. (19 g) was chromatographed on a silica gel column using the same solvent system of increasing polarity to give 14 frs. Zhankuic acid D (**2**, 50 mg), E (**5**, 15 mg) and dehyrdrosulphurenic acid (**8**, 20 mg) were obtained from frs 1, 5 and 6, respectively, by using prep. TLC plates. Dehydroburicoic acid (**7**, 50 mg) was furnished from fr. 3 by trituating the residue with $Me_2CO$ followed by recrystallization.

*Zhankuic acid D* (**2**). Pale yellow needle crystals, UV $\lambda_{max}^{MeOH}$: 244 nm (log ε 3.6); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 2976, 2938, 1733, 1712, 1650, 1459, 1378, 1375, 1239, 1189, 1183, 903; $^1$H NMR (300 MHz, $CDCl_3$): δ 1.40 (1H, H-1α), 3.04 (1H, H-1β), 2.42 (1H, H-2), 2.50 (1H, H-2), 2.43 (1H, H-4), 1.86 (1H, H-5), 2.4 (1H, H-6α), 2.5 (1H, H-6β), 2.38 (1H, *brd*, $J$ = 14 Hz, H-12α), 2.91 (1H, *d*, $J$ = 14 Hz, H-12β), 2.62 (1H, *dd*, $J$ = 11.9, 7.3 Hz, H-14), 1.4 (1H, H-15), 2.46 (1H, H-15), 1.2 (1H, H-16), 1.95 (1H, H-16), 1.4 (1H, H-17), 0.67 (3H, *s*, H-18), 1.50 (3H, *s*, H-19), 1.4 (1H, H-20), 0.91 (3H, *d*, $J$ = 5.3 Hz, H-21), 1.19 (1H, *m*, H-22), 1.55 (1H, H-22), 1.92 (1H, H-23), 2.13 (1H, *m*, H-23), 3.08 (1H, *q*, $J$ = 7.0 Hz, H-25), 1.23 (3H, *d*, $J$ = 7.0 Hz, H-27), 4.84 (1H, *brs*, H-28a), 4.89 (1H, *d*, $J$ = 4.0 Hz, H-28b), 1.01 (3H, *d*, $J$ = 6.6 Hz, H-29), 4.10 (2H, *q*, $J$ = 7.1 Hz, $OC\underline{H}_2CH_3$), 1.21 (3H, *t*, $J$ = 7.1 Hz, $OCH_2C\underline{H}_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 34.7 (*t*, C-1), 37.5 (*t*, C-2), 210.8 (*s*, C-3), 43.9 (*d*, C-4), 48.9 (*d*, C-5), 38.9 (*t*, C-6), 200.7 (*s*, C-7), 145.5 (*s*, C-8), 151.9 (*s*, C-9), 38.3 (*s*, C-10), 202.5 (*s*, C-11), 57.3 (*t*, C-12), 47.1 (*s*, C-13), 49.3 (*d*, C-14), 24.8 (*t*, C-15), 27.8 (*t*, C-16), 54.0 (*d*, C-17), 11.9 (*q*, C-18), 16.2 (*q*, C-19), 35.6 (*d*, C-20), 18.5 (*q*, C-21), 33.8 (*t*, C-22), 31.2 (*t*, C-23), 148.5 (*s*, C-24), 45.6 (*d*, C-25), 174.5 (*s*, C-26), 16.2 (*q*, C-27), 110.8 (*t*, C-28), 11.4 (*q*, C-29), 60.5 (*t*, $O\underline{C}H_2CH_3$), 14.2 (*q*, $OCH_2\underline{C}H_3$); EI-MS *m/z* (rel. int.): 496 (69), 482 (33), 468 (4), 450 (5), 422 (5), 354 (27), 341 (10), 326 (7), 313 (29), 311 (100), 286 (23), 273 (15), 260 (29), 246 (10), 220 (24), 205 (11), 189 (10), 175 (6), 142 (11), 123 (9), 109 (22), 102 (6), 95 (35), 82 (13), 68 (30).

*Zhankuic acid E* (**5**). Pale yellow needle crystals, UV $\lambda_{max}^{MeOH}$: 268 nm (log ε 3.7); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3387, 2955, 2926, 2869, 1730, 1668, 1460, 1377, 1329, 1186, 990, 973, 904; $^1$H NMR (300 MHz, $CDCl_3$): δ 3.77 (1H, *brs*, H-3), 4.04 (1H, *s*, H-12β), 2.99 (1H, *dd*, $J$ = 12.4, 7.4 Hz, H-14), 0.62 (3H, *s*, H-18), 1.29 (3H, *s*, H-19), 0.95 (3H, *d*, $J$ = 5.5 Hz, H-21), 3.09 (1H, *q*, $J$ = 7.0 Hz, H-25), 1.28 (3H, *d*, $J$ = 7.0 Hz, H-27), 4.84 (1H, *d*, $J$ = 3.9 Hz, H-28a), 4.89 (1H, *d*, $J$ = 3.9 Hz, H-28b), 0.93 (3H, *d*, $J$ = 6.3 Hz, H-29), 4.11 (2H, *q*, $J$ = 7.1 Hz, $OC\underline{H}_2CH_3$), 1.21 (3H, *t*, $J$ = 7.1 Hz, $OCH_2C\underline{H}_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 27.8 (*t*, C-1), 28.9 (*t*, C-2), 70.4 (*d*, C-3), 34.5 (*d*, C-4), 40.7 (*d*, C-5), 38.1 (*t*, C-6), 201.6 (*s*, C-7), 144.9 (*s*, C-8), 152.4 (*s*, C-9), 38.3 (*s*, C-10), 202.7 (*s*, C-11), 80.8 (*t*, C-12), 49.5 (*s*, C-13), 41.8 (*d*, C-14), 23.9 (*t*, C-15), 26.9 (*t*, C-16), 45.6 (*d*, C-17), 11.5 (*q*, C-18), 16.1 (*q*, C-19), 35.4 (*d*, C-20), 17.9 (*q*, C-21), 33.9 (*t*, C-22), 31.2, (*t*, C-23), 148.5 (*s*, C-24), 45.6 (*d*, C-25), 174.5 (*s*, C-26), 16.3 (*q*, C-27), 110.7 (*t*, C-28), 15.6 (*q*, C-29), 60.5 (*t*, $O\underline{C}H_2CH_3$), 14.2 (*q*, $OCH_2\underline{C}H_3$); EIMS *m/z* (rel. int.): 514 (100), 486 (3), 372 (3), 357 (4), 341 (10), 331 (9), 329 (18), 313 (16), 303 (24), 291 (29), 275 (21), 261 (21), 248 (17), 229 (8), 215 (7), 201 (10), 189 (11), 175 (17), 161 (13), 149 (19), 137 (22), 123 (10), 121 (25), 109 (45), 95 (13), 81 (13), 69 (11), 57 (7).

*Dehydroburicoic acid* (**7**). White powder, $[\alpha]_D^{25}$ + 75 ($c$ = 0.11, $CHCl_3$); UV $\lambda_{max}^{MeOH}$: 243 nm (log ε 4.12), 250 nm (log ε 3.98), 237 nm (log ε 4.04); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3435, 2961, 2875, 1719, 1703, 1655, 1459, 1377, 1225, 1194, 1076, 1031, 890; $^1$H NMR (300 MHz, pyridine-$d_5$): δ 1.90 (2H, H-2), 3.44 (1H, *t*, $J$ = 7.5 Hz, H-3), 1.28 (1H, H-5), 2.18 (2H, H-6), 5.63 (1H, *brs*, H-7), 5.39 (1H, *d*, $J$ = 5.4 Hz, H-11), 2.5 (1H, H-12α), 2.34 (1H, H-12β), 1.00 (3H, *s*, H-18), 1.12 (3H, *s*, H-19), 2.30 (1H, H-25), 1.06 (3H, H-26), 1.06 (3H, H-27), 1.06 (3H, *s*, H-29), 1.04 (3H, *s*, H-30), 1.03 (3H, *s*, H-31); (300 MHz, $CDCl_3/CD_3OD$): δ 3.04 (1H, *t*, $J$ = 7.9 Hz, H-3), 5.49 (1H, *brs*, H-7), 5.32 (1H, *d*, $J$ = 6.0 Hz, H-11), 2.08 (1H, H-12α), 1.62 (1H, *dd*, $J$ = 17.5, 6.0 Hz, H-12β), 0.47 (3H, *s*, H-18), 0.83 (3H, *s*, H-19), 0.96 (3H, *d*, $J$ = 6.8 Hz, H-26), 0.95 (3H, *d*, $J$ = 6.9 Hz, H-27), 4.51 (1H, *s*, H-28a), 4.59 (1H, *s*, H-28b), 0.70 (3H, *s*, H-29), 0.73 (3H, *s*, H-30), 0.80 (3H, *s*, H-31); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 36.5 (*t*, C-1), 28.5 (*t*, C-2), 78.1 (*d*, C-3), 39.4 (*s*, C-4), 49.1 (*d*, C-5), 23.6 (*t*, C-6), 121.3 (*d*, C-7), 142.9 (*s*, C-8), 146.7 (*s*, C-9), 37.9 (*s*, C-10), 116.7 (*d*, C-11), 36.1 (*t*, C-12), 44.4 (*s*, C-13), 50.6 (*s*, C-14), 31.9 (*t*, C-15), 27.3 (*t*, C-16), 48.2 (*d*, C-17), 16.3 (*q*, C-18), 23.4 (*q*, C-19), 49.9 (*d*, C-20), 178.3 (*s*, C-21), 32.8 (*t*, C-22), 31.7 (*t*, C-23), 156.0 (*s*, C-24), 34.3 (*d*, C-25), 22.0 (*q*, C-26), 22.1 (*q*, C-27), 107.1 (*t*, C-28), 26.0 (*q*, C-29), 28.7 (*q*, C-30), 16.6 (*q*, C-31); HREI-MS: 468.3596 ($C_{31}H_{48}O_3$, calcd 468.3603); EI-MS *m/z* (rel. int.): 468 (100), 455 (40), 437 (42), 372 (7), 341 (11), 311 (59), 297 (22), 281 (18), 271 (41), 253 (30), 240 (21), 225 (19), 213 (11), 201 (11), 187 (19), 173 (17), 159 (18), 135 (23), 121 (11), 109 (16), 97 (16), 83 (9), 71 (9), 57 (9), 43 (20), 44 (19).

*Dehydroeburicoic acid monoacetate* (**9**). Acetylation ($Ac_2O$–Py; 1:1; room temp. of **7** (30 mg) gave after work-up and column chromatogrpahy (silica gel) **9** (25 mg) as a solid, UV $\lambda_{max}^{MeOH}$: 243 nm (log $\varepsilon$ 4.02), 250 nm (log $\varepsilon$ 3.91), 237 nm (log $\varepsilon$ 3.96); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3449, 2960, 1767, 1717, 1678, 1655, 1648, 1459, 1377, 1249, 1034, 893, 815; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$1.69 (2H, H-2), 4.48 (1H, *dd*, $J$ = 8.9, 6.4 Hz, H-3), 1.20 (1H, H-5), 2.05 (2H, H-6), 5.46 (1H, *brs*, H-7), 5.27 (H, *d*, $J$ = 6.1 Hz, H-11), 2.16 (1H, H-12$\alpha$), 1.82 (1H, *dd*, $J$ = 16.1, 6.1 Hz, H-12$\beta$), 0.62 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 2.20 (1H, H-25), 1.00 (3H, *d*, $J$ = 6.8 Hz, H-26), 0.99 (3H, *d*, $J$ = 6.9 Hz, H-27), 4.67 (1H, *s*, H-28a), 4.74 (1H, *s*, H-28b), 0.86 (3H, *s*, H-29), 0.88 (3H, *s*, H-30), 0.93 (3H, *s*, H-31), 2.03 (3H, *s*, $COCH_3$); $^{13}C$ NMR $\delta$ (75 MHz, $CDCl_3$): 35.4 (*t*, C-1), 24.3 (*t*, C-2), 80.6 (*d*, C-3), 37.3 (*s*, C-4), 49.3 (*d*, C-5), 22.8 (*t*, C-6), 120.5 (*d*, C-7), 142.3 (*s*, C-8), 145.8 (*s*, C-9), 37.6 (*s*, C-10), 116.3 (*d*, C-11), 35.6 (*t*, C-12), 43.6 (*s*, C-13), 50.1 (*s*, C-14), 31.0 (*t*, C-15), 26.9 (*t*, C-16), 47.5 (*d*, C-17), 15.8 (*q*, C-18), 22.7 (*q*, C-19), 47.6 (*d*, C-20), 181.6 (*s*, C-21), 30.9 (*t*, C-22), 32.0 (*t*, C-23), 155.2 (*s*, C-24), 33.8 (*d*, C-25), 21.8 (*q*, C-26), 21.8 (*q*, C-27), 106.9 (*t*, C-28), 25.6 (*q*, C-29), 28.1 (*q*, C-30), 16.9 (*q*, C-31), 170.9 (*s*, O<u>C</u>OCH$_3$), 21.3 (*q*, OCO<u>C</u>H$_3$); EIMS *m/z*: (rel. int.): 510 (48), 497 (16), 450 (10), 437 (57), 407 (3), 381 (4), 367 (5), 353 (85), 339 (18), 331 (6), 313 (25), 301 (14), 295 (34), 288 (21), 281 (27), 271 (10), 253 (100), 240 (44), 225 (42), 213 (22), 201 (17), 187 (36), 169 (35), 159 (31), 145 (19), 135 (29), 121 (21), 109 (33), 97 (39), 91 (5), 84 (20), 71 (11), 57 (12), 43 (20).

*Dehydrosulphurenic acid* (**8**). White powder, mp 240–247°, $[\alpha]_D^{25}$ + 55 ($c$ = 0.1, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ 243 nm (log $\varepsilon$ 4.07), 250 nm (log $\varepsilon$ 3.95), and 237 nm (log $\varepsilon$ 3.99); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2962, 2932, 1773, 1699, 1686, 1458, 1377, 1273, 1048, 889; $^1H$ NMR $\delta$ (300 MHz, pyridine-$d_5$): 1.90 (2H, H-2), 3.44 (1H, *m*, H-3), 1.30 (1H, H-5), 2.16 (2H, H-6), 5.50 (1H, *brs*, H-7), 5.38 (1H, *d*, $J$ = 5.3 Hz, H-11), 2.70 (1H, H-12$\alpha$), 2.37 (1H, H-12$\beta$), 4.80 (1H, *m*, H-15), 1.70 (1H, H-16), 2.37 (1H, H-16), 1.08 (3H, *s*, H-18), 1.10 (3H, *s*, H-19), 2.22 (1H, H-25), 0.99 (3H, *d*, $J$ = 7.0 Hz, H-26), 0.98 (3H, *d*, $J$ = 6.9 Hz, H-27), 4.84 (1H, *s*, H-28a), 4.88 (1H, *s*, H-28b), 1.44 (3H, *s*, H-29), 1.17 (3H, *s*, H-30), 1.13 (3H, *s*, H-31); $^{13}C$ NMR (75 MHz, pyridine-$d_5$): $\delta$ 36.9 (*t*, C-1), 28.9 (*t*, C-2), 78.1 (*d*, C-3), 39.4 (*s*, C-4), 49.7 (*d*, C-5), 23.6 (*t*, C-6), 122.3 (*d*, C-7), 142.0 (*s*, C-8), 147, 1 (*s*, C-9), 38.0 (*s*, C-10), 116.3 (*d*, C-11), 36.4 (*t*, C-12), 44.9 (*s*, C-13), 52.5 (*s*, C-14), 73.8 (*t*, C-15), 39.6 (*t*, C-16), 46.5 (*d*, C-17), 16.9 (*q*, C-18), 23.1 (*q*, C-19), 48.9 (*d*, C-20), 178.7 (*s*, C-21), 32.7 (*t*, C-22), 31.9 (*t*, C-23), 155.8 (*s*, C-24), 34.2 (*d*, C-25), 21.9 (*q*, C-26), 22.0 (*q*, C-27), 107.1 (*t*, C-28), 18.3 (*q*, C-29), 28.7 (*q*, C-30), 16.7 (*q*, C-31); HREI-MS: 484.3520 ($C_{31}H_{48}O_4$, calcd 484.3553); EI-MS *m/z* (rel. int.): 484 (100), 470 (13), 468 (10), 451 (22), 433 (10), 423 (12), 400 (9), 383 (6), 367 (6), 351 (18), 339 (9), 327 (24), 311 (25), 295 (10), 285 (10), 273 (16), 261 (10), 239 (9), 227 (13), 221 (21), 211 (8), 199 (7), 179 (10), 159 (11), 147 (8), 135 (9), 123 (11), 107 (11), 97 (11), 83 (8), 69 (5), 44 (8).

*15$\alpha$-Acetyl-dehydrosulphurenic acid* (**6**). White powder, mp 243–248°, $[\alpha]_D^{25}$ + 177 ($c$ = 0.1, $CHCl_3$); UV $\lambda_{max}^{MeOH}$: 243 nm (log $\varepsilon$ 4.13), 250 nm (log $\varepsilon$ 3.98), 237 nm (log $\varepsilon$ 4.04); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3435, 2961, 2932, 1735, 1718, 1655, 1459, 1378, 1249, 1039, 993, 889; $^1H$ NMR (300 MHz, $CDCl_3$). $\delta$ 1.66 (2H, H-2), 3.22 (1H, *dd*, $J$ = 11, 4.6 Hz, H-3), 1.06 (1H, H-5), 2.06 (2H, H-6), 5.50 (1H, *d*, $J$ = 6.1 Hz, H-7), 5.26 (1H, *d*, $J$ = 6.2 Hz, H-11), 2.20 (1H, H-12$\alpha$), 1.80 (1H, *dd*, $J$ = 17, 6.2 Hz, H-12$\beta$), 5.04 (1H, *dd*, $J$ = 9.6, 5.5 Hz, H-15), 1.72 (1H, H-16), 2.10 (1H, H-16), 1.08 (3H, *s*, H-18), 1.10 (3H, *s*, H-19), 2.14 (1H, H-25), 0.99 (3H, *d*, $J$ = 7.0 Hz, H-26), 0.98 (3H, *d*, $J$ = 6.9 Hz, H-27), 4.74 (1H, *s*, H-28a), 4.64 (1H, *s*, H-28b), 0.93 (3H, *s*, H-29), 0.85 (3H, *s*, H-30), 1.00 (3H, *s*, H-31), 2.08 (3H, *s*, OAc); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$35.6 (*t*, C-1), 27.8 (*t*, C-2), 78.8 (*d*, C-3), 38.6 (*s*, C-4), 48.8 (*d*, C-5), 23.0 (*t*, C-6), 121.8 (*d*, C-7), 139.9 (*s*, C-8), 146.0 (*s*, C-9), 37.4 (*s*, C-10), 115.6 (*d*, C-11), 29.7 (*t*, C-12), 43.9 (*s*, C-13), 51.0 (*s*, C-14), 76.7 (*t*, C-15), 35.8 (*t*, C-16), 45.6 (*d*, C-17), 16.2 (*q*, C-18), 22.7 (*q*, C-19), 47.2 (*d*, C-20), 181.5 (*s*, C-21), 35.9 (*t*, C-22), 31.9 (*t*, C-23), 154.8 (*s*, C-24), 33.7 (*d*, C-25), 21.7 (*q*, C-26), 21.8 (*q*, C-27), 107.0 (*t*, C-28), 18.5 (*q*, C-29), 28.2 (*q*, C-30), 15.8 (*q*, C-31), 21.4 (*q*, CO<u>C</u>H$_3$), 171.2 (*s*, <u>C</u>OCH$_3$); HREI-MS: 526.3668 ($C_{33}H_{51}O_5$, calcd 526.3658); EIMS *m/z* (rel. int.): 526 (98), 513 (12), 469 (28), 453 (39), 435 (48), 372 (20), 353 (17), 339 (14), 326 (15), 313 (46), 311 (100), 295 (48), 259 (53), 241 (38), 225 (32), 211 (22), 199 (22), 187 (28), 171 (29), 159 (22), 145 (16), 133 (13), 121 (15), 111 (15), 97 (21), 83 (10), 44 (10).

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