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TWO DIURETIC TRITERPENOIDS FROM *ANTIDESMA MENASU**

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Key Word Index *Antidesma menasu*; Euphorbiaceae; aerial portion; triterpenes; 16α-hydroxy-3-ketoisomultiflorene; 3β-hydroxy-16-ketoisomultiflorene; structural analysis; diuretic activity.

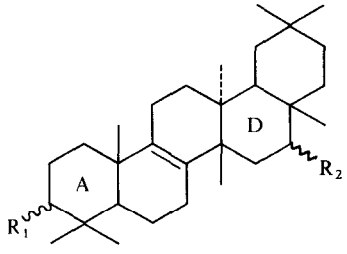
Abstract—Two new pentacyclic triterpenoids characterized as 16α-hydroxy-3-ketoisomultiflorene and 3β-hydroxy-16-ketoisomultiflorene have been isolated from the aerial parts of *Antidesma menasu*. Both of these compounds displayed diuretic activity in experimental animals.

INTRODUCTION

Separation of individual components from the biologically active terpenoid fraction [1] obtained from the aerial parts of *Antidesma menasu* Miq. ex. Tul. (Euphorbiaceae) and their rigorous purification utilizing column chromatography and preparative TLC on 10% $AgNO_3$-impregnated Si gel resulted in the isolation of two new isomeric triterpenes. Their structural elucidation is described in the present communication.

RESULTS AND DISCUSSION

The triterpene (**1**), mp 294–295° ($CHCl_3$–MeOH); $[\alpha]_D^{25}$ + 67° (*c*, 1, $CHCl_3$); ν_{max}^{KBr} 3450 (OH) and 1700 cm^{-1} (C=O) showed a molecular ion at *m/e* 440 in its MS corresponding to the molecular formula $C_{30}H_{48}O_2$. This, coupled with the presence of eight methyl singlets (δ 0.65–1.18) and one hydroxymethine multiplet (3.70) in its ^{1}H NMR spectrum suggested it possessed a pentacyclic triterpenoid skeleton. Deshielding of the expected magnitude experienced by the hydroxymethine (δ 4.78) in its acetate (**2**), mp 234–236°; M^+ at *m/e* 482; $[\alpha]_D^{25}$ + 35° (*c*, 1, $CHCl_3$); demonstrated the secondary nature of the hydroxy function. There were no signals for olefinic protons in the ^{1}H NMR spectrum of **1**. However, it responded to the tetranitromethane test revealing the presence of a tetra-substituted double bond which resisted catalytic hydrogenation under a variety of conditions. Further insight into its structure was obtained by the study of the EI mass spectral behaviour of **1** and its various derivatives. Identification of the fragment ions at *m/e* 425, 257, 245, 229 and 218 suggested it to have an isomultiflorene skeleton [2–4], thus accounting for the sluggish behaviour of the double bond situated at Δ^8. The occurrence of fragment ions at *m/e* 257, 245, 229, 218, 203, 151 and 135 in **1** and at *m/e* 263, 257, 245, 221, 203 and 135 in **2** required the placement of keto and hydroxy functions in rings A and D, respectively. Further, **1**, on Huang–Minlon reduction followed by oxidation, afforded 16-ketoisomultiflorene (**3**) [5], mp 210°; M^+ at *m/e* 424. This confirmed the placement of the hydroxy function in **1** at C-16. Compound **1** was oxidized with Corey's reagent to a dione (**4**), mp 250–252°; M^+ at *m/e* 438; $[\alpha]_D^{25}$ + 10° (*c*, 1, $CHCl_3$); which on reduction with Na/isoamyl alcohol gave a diol (**5**), mp 175–179°; M^+ at *m/e* 442. As the same diol (**5**) was obtained by direct reduction of **1** under similar conditions, the hydroxy at C-16 was inferred to have an α-orientation.



	R_1	R_2
1	=O	α-OH, β-H
2	=O	α-OAc, β-H
3	H	=O
4	=O	=O
5	α-OH, β-H	α-OH, β-H
6	β-OH, α-H	=O
7	β-OAc, α-H	=O
8	β-OH, α-H	H

The triterpene **6**, mp 275–278° ($CHCl_3$–MeOH); M^+ at *m/e* 440; $C_{30}H_{48}O_2$; $[\alpha]_D^{25}$ + 33° ($CHCl_3$), ν_{max}^{KBr} 3450 (OH) and 1710 cm^{-1} (C=O) had eight methyl singlets (δ 0.75–1.04) and a hydroxymethine multiplet (3.80) similar to compound **1** in its ^{1}H NMR spectrum. An examination of the cracking pattern of **6** under EI (*m/e* 259, 247, 241, 220, 219, 191, 149, 133 and 121) and that of its acetate (**7**), mp 255–258°, M^+ at *m/e* 482; $[\alpha]_D^{25}$ + 40° ($CHCl_3$) (*m/e* 301, 289, 259, 247, 219, 149 and 133) indicated a striking resemblance to compound **1** with the hydroxy and keto functionalities placed in rings A and D,

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respectively. Compound **6** was converted under Huang-Minlon reduction conditions to isomultiflorenol (**8**) [6] confirming a β-OH at C-3. Since both the triterpenes gave an identical oxidation product (**4**) the carbonyl was established at C-3 in **1** and at C-16 in **6** thus elucidating their complete structures as 16α-hydroxy-3-ketoisomultiflorene and 3β-hydroxy-16-ketoisomultiflorene, respectively.

On preliminary screening, compounds **1** and **6** exhibited diuretic activity in rats to the extent of 79% that of chlorothiazide at a dose level of 125 mg/kg.

EXPERIMENTAL

All mps are uncorr. The ^{1}H NMR spectra were recorded at 90 MHz using HMDS as an internal standard. The MS were taken with a direct inlet system.

Isolation of compounds **1** *and* **6**. The dried, powdered aerial portion of *A. menasu* (6 kg) was percolated with *n*-hexane (4 × 7 l.) and the extract concd *in vacuo* to give a white solid (8 g) consisting of a mixture of triterpenes. Compounds **1** and **6** were separated from the terpene mixture using repeated CC and prep. TLC over 10% $AgNO_3$-impregnated Si gel.

16α-*Hydroxy*-3-*ketoisomultiflorene* (**1**). 300 mg eluted with a mixture of $CHCl_3$–MeOH (99:1) and purified by prep. TLC ($CHCl_3$–MeOH, 98:2) had mp 294–295° ($CHCl_3$–MeOH); $[\alpha]_D^{25}$ + 67° (*c*, 1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3450, 2900, 1700, 1440 and 1380; ^{1}H NMR ($CDCl_3$): δ 0.65–1.18 (8*s*, 24H, 8 × C$\underline{H}_3$) and 3.70 (*m*, 1H, –C$\underline{H}$OH); MS *m/e*: 440 (M^+), 425, 422, 257, 245, 229, 218, 203, 151 and 135.

16α-*Acetoxy*-3-*ketoisomultiflorene* (**2**). A mixture of **1** (50 mg), Ac_2O (1 ml) and pyridine (2.5 ml) was left overnight at room temp. The usual work-up followed by purification by prep. TLC yielded white silky needles (40 mg), mp 234–236° ($CHCl_3$–MeOH), $[\alpha]_D^{25}$ + 35° (*c*, 1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2900, 1720, 1710, 1440 and 1240; ^{1}H NMR ($CDCl_3$): δ 0.67–1.2 (7*s*, 24H, 8 × C$\underline{H}_3$), 1.97 (*s*, 3H, –OCOC$\underline{H}_3$) and 4.78 (*m*, 1H, –C$\underline{H}$OAc); MS *m/e*: 482 (M^+), 467, 454, 440, 425, 263, 257, 245, 221, 203 and 135.

16-*Ketoisomultiflorene* (**3**). A mixture of **1** (50 mg), KOH (100 mg), diethylene glycol (5 ml) and hydrazine hydrate (99%, 1 ml) was refluxed at 180° for 1.5 hr and the temp. gradually raised to 220°. After refluxing for another 3 hr it was worked up to yield a residue (40 mg) which was purified by prep. TLC to afford 16α-hydroxyisomultiflorene. The latter on oxidation with Corey's reagent gave **3** (35 mg), mp 210° ($CHCl_3$–MeOH); IR ν_{max}^{KBr} cm^{-1}: 2900, 1710, 1440 and 1380; MS *m/e*: 424 (M^+), 409, 231, 219, 218, 191, 180, 149, 133 and 121.

3,16-*Diketoisomultiflorene* (**4**). A soln of **1** or **6** (100 mg) in $CHCl_3$ was stirred with Corey's reagent (200 mg) for 1 hr at room temp. After the completion of the reaction the solvent was evapd off and the residue chromatographed over a column of Si gel. The eluent from $CHCl_3$–MeOH (99:1) gave **4** (~60 mg), mp 250–252°; $[\alpha]_D^{25}$ + 10° (*c*, 1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2900, 1730, 1700, 1440 and 1380; MS *m/e*: 438 (M^+), 423, 257, 229, 219, 191, 149 and 133. (Found: C, 84.6; H, 11.65. $C_{30}H_{46}O_2$ requires: C, 84.9; H, 11.32%).

3α,16α-*Dihydroxyisomultiflorene* (**5**). The reaction of **1** or **4** (50 mg) with Na (500 mg) in isoamyl alcohol (5 ml) gave **5** (~35 mg), mp 175–179°; IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1460, 1380, 1260 and 1020; MS *m/e*: 442 (M^+), 424, 409, 259, 247, 229, 221, 203, 191, 177, 163, 151 and 135.

3β-*Hydroxy*-16-*ketoisomultiflorene* (**6**). 300 mg eluted with a mixture of $CHCl_3$–MeOH (99:1) and purified by prep. TLC had mp 275–278° ($CHCl_3$–MeOH); $[\alpha]_D^{25}$ + 33° (*c*, 1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3450, 2900, 1710, 1440, 1380 and 990; ^{1}H NMR ($CDCl_3$): δ 0.75–1.04 (8*s*, 24H, 8 × C$\underline{H}_3$) and 3.80 (*m*, 1H, –C$\underline{H}$OH); MS *m/e*: 440 (M^+), 425, 422, 259, 247, 229, 219, 205, 191, 149 and 133.

3β-*Acetoxy*-16-*ketoisomultiflorene* (**7**). A mixture of **6** (50 mg), Ac_2O (1 ml) and pyridine (2.5 ml) was left overnight at room temp. The usual work-up yielded white needles (40 mg), mp 255–258° ($CHCl_3$–MeOH); $[\alpha]_D^{25}$ + 40° (*c*, 1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2900, 1720, 1710, 1440, 1380 and 1240; ^{1}H NMR ($CDCl_3$): δ 0.7–1.3 (8*s*, 24H, 8 × C$\underline{H}_3$), 2.07 (*s*, 3H, –OCOC$\underline{H}_3$) and 4.78 (*m*, 1H, –C$\underline{H}$OAc); MS *m/e*: 482 (M^+), 467, 440, 301, 289, 259, 247, 230, 229, 219, 191, 149, 133 and 121.

Isomultiflorenol (**8**). A mixture of **6** (50 mg), KOH (100 mg), diethylene glycol (5 ml) and hydrazine hydrate (99%, 1 ml) was subjected to Huang-Minlon reduction conditions to give **8** (30 mg), mp 175–176° ($CHCl_3$–MeOH); $[\alpha]_D^{25}$ + 22° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3450, 2900, 1450 and 1380; MS *m/e*: 426 (M^+), 411, 393, 259, 247, 229, 205, 191, 149 and 133.

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REFERENCES

1. Rizvi, S. H., Shoeb, A., Kapil, R. S. and Popli, S. P. (1980) *Experientia* **36**, 146.
2. Nishimoto, K., Ito, M., Natori, S. and Ohmoto, T. (1968) *Tetrahedron* **24**, 735.
3. Talapatra, S. K., Sengupta, S. and Talapatra, B. (1968) *Tetrahedron Letters* 5963.
4. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
5. Kikuchi, T., Niwa, M., Takayama, M., Yokoi, T. and Shingu, T. (1973) *Tetrahedron Letters* 1987.
6. Sengupta, P. and Khastgir, H. N. (1963) *Tetrahedron* **19**, 123.