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AN ARTEMISINIC ACID ANALOGUE FROM *TITHONIA DIVERSIFOLIA*

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Key Word Index—*Tithonia diversifolia*; compositae; stem, artemisinic acid analogue.

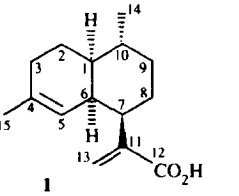
Abstract—A new artemisinic acid analogue compound has been isolated from mature stems of *Tithonia diversifolia* and its structure determined.

INTRODUCTION

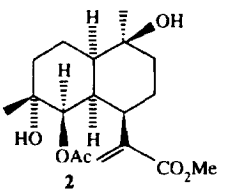
The discovery of artemisinin, a potent antimalarial drug isolated from the Chinese traditional medicinal plant, *Artemisia annua*, and the co-occurrence of artemisinic acid **1**, the biogenetic precursor of artemisinin [1, 2], has intensified the search for other analogues of artemisinin, as well as artemisinic acid, in the Compositae in general and in other *Artemisia* species in particular. In continuation of our earlier work on *Tithonia diversifolia*, from which we isolated several antifeedant sesquiterpene lactones [3, 4], we investigated mature stems of this species and isolated an ester **2**, related to artemisinic acid **1**. The structure of this ester has been established through extensive use of ^{1}H and ^{13}C NMR spectrometry and chemical transformations. Compound **2** has functionalities suitable for further conversion to 1,2,4-trioxane-type artemisinin analogues [5–7].

RESULTS AND DISCUSSION

The chloroform extract of air-dried mature stems after separation of **3** and **4**, gave by further thin layer chromatographic separation on silica gel, a crystalline compound A, $C_{18}H_{28}O_6$ by HR-mass spectrometry with a $[M]^+$ at *m/z* 340. Other important peaks were observed at *m/z* 323, 322, 307, 291, 280, 262, etc. The IR spectrum showed characteristic bands at 3500 (–OH stretching), 1725 (–$OCOCH_3$ stretching), 1685 and 1625 cm^{-1} (α,β-unsaturated acid). In the ^{1}H NMR spectrum, four singlets at δ0.8, 1.15, 2.0 and 3.6, each integrating for three protons revealed the presence of two tertiary methyls, one acetoxyl methyl and one ester methyl, respectively. A broad one-proton singlet at δ4.6 was attributed to the proton of the acetoxyl group. Two broad one-proton singlets at δ5.4 and 6.0 were assigned to an exocyclic methylene of an α,β-unsaturated acid. The broad-band decoupled ^{13}C NMR spectrum showed the presence of 18 carbons, three of which were quaternary, three methines, five methylenes and four methyl carbons as revealed by the off-resonance ^{13}C NMR spectrum.



1



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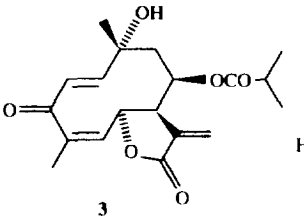

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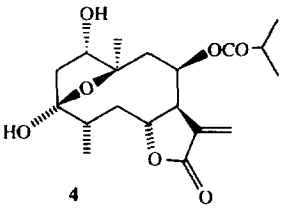

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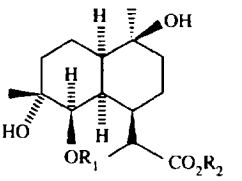
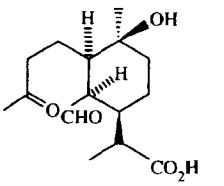


	R_1	R_2
5	Ac	Me
6	H	H



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Hydrogenation of A under atmospheric pressure using platinum oxide catalyst, gave a dihydro compound **B**, as revealed by its mass spectrum. The IR spectra showed characteristic bands at 3500, 2950, 2850 and 1725 cm^{-1}. The ^{1}H NMR spectrum showed the absence of the two broad singlets at δ5.4 and 6.0 present in the ^{1}H NMR spectrum of A and the appearance of a three proton doublet at δ1.2 with J = 7 Hz. Four three proton singlets at δ0.8, 1.1, 2.05, and 3.65, were assigned to two tertiary methyls, one acetoxyl and one ester methyl group, respectively. The dihydro derivative was hydrolysed by sodium methoxide to give a trihydroxy acid $C_{15}H_{26}O_5$ whose IR spectrum revealed the presence of hydroxyl

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groups (3550 cm^{-1}) and a carboxylic acid group (1725 cm^{-1}). The ^1H NMR spectrum contained a three-proton doublet at δ1.2 (J = 7 Hz), two three-proton singlets at δ0.8 and 1.05 and two broad one-proton singlets at δ3.6 and 11.5. The hydrolysed compound was easily cleaved to a ketoaldehydo carboxylic acid ($C_{15}H_{24}O_5$) by activated manganese dioxide in dichloromethane, thus showing the presence of a tertiary–secondary vicinal diol system. The IR spectrum revealed the presence of hydroxyl groups (3550 cm^{-1}), overlapping strong bands of a carboxylic acid group and an aldehyde group (1725 cm^{-1}), and a ketone (1710 cm^{-1}). The ^1H NMR spectrum showed the presence of one methyl doublet at δ1.1 (J = 7 Hz), two methyl singlets at δ0.95 and 2.2, one broad two-proton singlet at δ3.5, a broad one-proton singlet at δ11.5 and a one-proton doublet at δ9.6. These results led to structures **5**, **6** and **7** for compound B, trihydroxy acid and the ketoaldehydo carboxylic acid, respectively. Therefore, the gross structure of the parent compound A could be expressed as **2**. The ^{13}C NMR data of A also fully supported structure **2** for this compound.

The relative stereochemistry of **2** was assigned on the basis of coupling constants obtained by decoupling experiments. Since a *cis*-decalin can exist in two conformation, it is clear from the coupling constants $J_{5,6}$ = 2.0 Hz and $J_{1,6}$ = 4 Hz that H-6 is equatorial and H-1 is axial. In the presence of trifluoroacetyl isocyanide, the signals of H-5 and H-6 were shifted to δ5.30 and 3.10, respectively, indicating that the hydroxyl and the acetoxyl group at C-4 and C-5 are α and β, respectively, and also that H-6 is α. Since the H-1 signal was not appreciably shifted, it was inferred that the hydroxyl at C-10 is β.

EXPERIMENTAL

General. Mps: uncorr. IR: KBr or neat film. UV: EtOH. $[\alpha]_D$: $CHCl_3$. ^1H NMR: 270 MHz; ^{13}C NMR: 75 MHz; $CDCl_3$ with TMS as int. standard. EIMS at 70 eV. Petrol refers to bp 60–80° fr.

Isolation of methyl 5-acetoxy-4,5-dihydro-4,10-dihydroxy-5-acetoxyartemisinate **2**. Air-dried mature stems of *T. diversifolia* Hemsl. A. Grey (2 kg) collected in the Bokakhat area of the Golaghat District, Assam, India, were extracted with $CHCl_3$ in a Soxhlet apparatus until the extract was colourless (*ca* 8 h). The crude material, after evapn of $CHCl_3$ under red. pres., was dissolved in MeOH (400 ml) containing 10% H_2O and left overnight. The ppt. was filtered and the filtrate washed with petrol (8 × 200 ml). The MeOH layer was concd under red. pres. and then extracted with $CHCl_3$. The washed and dried extract after evapn yielded 10 g of a crude gum which was chromatographed over 200 g of silica gel (60–120 mesh) and 200 ml frs were collected in the following order: frs 1–5 (petrol), 6–10 (petrol–EtOAc, 9:1), 11–16 (petrol–EtOAc, 4:1), 17–21 (petrol–EtOAc, 2:1), 22–28 (petrol–EtOAc), 1:1), 19–32 (petrol–EtOAc, 1:2), 33–38 (petrol–EtOAc, 1:4), 39–41 (EtOAc). Frs 11–16 showed several very minor compounds on TLC which were not investigated further. Frs 28–30 showed a minor spot on TLC which was identified by spectroscopic means as tagitinin C **3** reorted by us earlier from the leaves of this species. Similarly frs 35–40 contained another minor compound which was identified by spectroscopic means as tagitinin A **4**, another compound reported by us earlier from *T. diversifolia* [2].

Frs 17–25 showed a major spot on TLC (petrol–EtOAc, 1:9) which were combined to give *ca* 900 mg of crystalline methyl 5-acetoxy-4,5-dihydro-4,10-dihydroxy-5-acetoxyartemisinate **2** (0.45%). Mp 170° (petrol–EtOAc, 1:1). UV λ_{max} 220 nm (ε = 10,000). IR ν_{max} cm^{-1}: 3500, 2925, 2850, 1710, 1625, 1440. ^1H NMR: δ0.8 (3H, *s*, H-14), 1.15 (3H, *s*, H-15), 2.0 (3H, *s*, CH_3COO), 2.3 (1H, *ddd*, J = 2.5, 4 and 4.5 Hz, H-1), 2.6 (1H, *ddd*, J = 2, 4 and 4 Hz, H-6), 3.6 (3H, *s*, COOMe), 4.6 (1H, *dq*, J = 2 and 1 Hz, H-5), 5.4 (1H, *br s*, H-13a), 6.0 (1H, *br s*, H-13b). ^{13}C NMR: δ173.1 (*s*, acetoxy CO), 171.7 (*s*, C-12), 130.8 (*s*, C-11), 128.8 (*t*, C-13), 119.4 (*d*, C-5), 77.5 (*s*, C-4), 60.1 (*s*, C-10), 48.9 (*d*, C-6), 43.8 (*d*, C-1), 41.4 (*d*, C-7), 38.9 (*t*, C-3), 34.4 (*t*, C-9), 31.8 (*t*, C-8), 30.5 (*t*, C-2), 29.6 (*q*, acetoxy methyl), 28.9 (*q*, ester methyl), 26.9 (*q*, C-15), 25.8 (*q*, C-14). MS *m/z*: 340 $[M]^+$, 323, 322, 307, 291, 280, 262, 249, 246, 223, 191, 187, 181, 145, 135, 121, 119, 107. (Found: C, 63.52; H, 8.63; $C_{18}H_{28}O_6$ requires C, 63.53; H, 8.64%).

5-Acetoxy-4,5-dihydroxy-4,5,11,13-tetrahydroartemisinic acid methyl ester **5**. A soln of 50 mg (0.15 mmol) of Me 6-acetoxy-4,5-dihydro-4,10-dihydroxy-5-acetoxyartemisinate **2** in 20 ml of MeOH was hydrogenated at room temp. and atm. pres. using platinum oxide (50 mg) as catalyst. After 6 hr, the catalyst was filtered off and washed with MeOH (5 ml × 3). The filtrate was evapd under red. pres. to obtain a crude mass which on purification by prep. TLC on silica gel afforded 40 mg of **5**. IR ν_{max} cm^{-1}: 3500, 2950, 2850, 1725, 1450, 1380. ^1H NMR: δ0.8 (3H, *s*, H-14), 1.1 (3H, *s*, H-15), 1.2 (3H, *d*, J = 7 Hz, H-13), 2.05 (3H, *s*, CH_3COO), 3.65 (3H, *s*, COOMe), 4.75 (1H, *br s*, H-5). MS *m/z*: 342 $[M]^+$, 324, 309, 293, 282, 264, 249, 223, 193, 161, 123, 119, 109, 107, 105. (Found: C, 63.11; H, 8.74; $C_{18}H_{30}O_6$ requires C, 63.15; H, 8.77%).

4,5,6-Trihydroxy-4,5,11,13-tetrahydroartemisinic acid **6**. A soln of 100 mg (0.35 mmol) of 6-acetoxy-4,5-dihydroxy-4,5,11,13-tetrahydro-artemisinic acid Me ester **5** in 5 ml of MeOH was treated with freshly prepd NaOMe (from 150 mg (6.50 mmol) of Na in 3 ml dry MeOH) and the reaction mixt. stirred at 20° monitoring the reaction by TLC. When no starting material remained, the reaction mixt. was dild with 200 ml of H_2O, neutralized with HOAc and then extracted with EtOAc (100 ml × 5). The dried extract was evapd under red. pres. and HOAc removed by co-distilling with toluene under vacuum. Purification by prep. TLC (petrol–EtOAc, 1:1) furnished 70 mg of **6**. Mp 198° (EtOAc). IR ν_{max} cm^{-1}: 3550, 2950, 2850, 1725, 1650, 1450, 1375. ^1H NMR: δ0.8 (3H, *s*, H-14), 1.05 (3H, *s*, H-15), 1.2 (3H, *d*, J = 7 Hz, H-13), 3.6 (1H, *br s*, H-5), 11.5 (1H, *br s*, COOH). MS *m/z*: 286 $[M]^+$, 269, 252, 235, 224, 207, 193, 183, 177, 175, 163, 133 and 107. (Found: C, 62.91; H, 9.05; $C_{15}H_{26}O_5$ requires C, 62.93; H, 9.09%).

Ketoaldehydocarboxylic acid **7**. To a soln of 70 mg (0.23 mmol) of 4,5,6-trihydroxy-4,5,11,13-tetrahydro-artemisinic acid **6** in dry CH_2Cl_2, was added 100 mg

(1.15 mmol) of activated MnO_2 (freshly prepd [8]) and the reaction mixt. stirred continuously until no starting material could be detected by TLC. The reaction mixt. was then filtered and the residue washed with CH_2Cl_2 (5 ml × 4). The combined organic phases were evapd under red. pres. to give a crude product, which on purification by prep. TLC gave a gummy mass, yield 57 mg. IR ν_{max} cm^{-1}: 2900, 2850, 1725, 1710, 1450, 1375, 1350. ^1H NMR: δ0.95 (3H, *s*, H-14), 1.1 (3H, *d*, J = 7 Hz, H-13), 2.2 (3H, *s*, H-15), 3.5 (overlapping signals H-3 and H-6), 9.8 (1H, *d*, J = 7 Hz, CHO at H-5), 11.5 (1H, *br s*, COOH). MS *m/z*: 296 $[M]^+$, 282, 280, 265, 223, 206, 177, 133, 107, 95, 88 and 81. (Found: C, 63.36; H, 8.43; $C_{15}H_{24}O_5$ requires C, 63.38; H, 45%).

REFERENCES

1. Zaman, S. S. and Sharma, R. P. (1991) *Heterocycles* **32**, 1593.
2. Bordoloi, M., Shukla, V. S., Nath, S. C. and Sharma, R. P. (1989) *Phytochemistry* **32**, 3007.
3. Barua, N. C., Sharma, R. P., Madhusudanan, K. P., Thyagarajan, G., Herz, G. and Murari, R. (1979) *J. Org. Chem.* **44**, 1831.
4. Dutta, P., Bhattacharyya, P. R., Radha, L. C., Borodoloi, D. N., Barua, N. C., Chowdhury, P. K., Sharma, R. P. and Baruah, J. N. (1986) *Phytoparasitica* **14**, 77.
5. Wei-Shan, Z., Lian, Z., Zhao-Chang, F. and Xing-Xiang, X. (1986) *Tetrahedron* **42**, 4437.
6. Schmid, G. and Hofheinz, W. (1983) *J. Am. Chem. Soc.* **105**, 624.
7. Zaman, S. S., Debnath, J., Sarmah, P., Barua, N. C. and Sharma, R. P. (1992) *J. Chem. Soc., Chem. Commun.* 329.
8. Attenburrow, J., Cameron, A. F. B., Chapman, J. H., Evans, R. M., Hems, B. A., Jansen, A. B. A. and Walker, T. (1952) *J. Chem. Soc.* 1094.