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MALLOTIN—A NEW C_{32} TRITERPENOID FROM *MALLOTOUS STENANTHUS*

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Key Word Index—*Mallotous stenanthus*; Euphorbiaceae; mallotin; lanostane-type triterpene.

Abstract—Mallotin, a new C_{32} triterpene from *Mallotous stenanthus*, has been isolated and its structure established as 24,24-dimethyl-lanosta-7,25-dien-3α-ol.

INTRODUCTION

Various species of *Mallotous* have been found to possess different types of biological activity [1, 2] and chemical investigations have resulted in the isolation of cardenolides [3, 4], red pigments [5], terpenoids [6, 7] and fatty acids [8]. Recently an alcoholic extract of *M. stenanthus* was found to exhibit significant anticancer activity [9] in the PS system in mice. Consequently, a detailed chemical examination has been undertaken.

RESULTS AND DISCUSSION

The alcohol extract was solvent fractionated and the butanol-soluble fraction showed the anticancer activity. This fraction was purified by treatment with lead hydroxide and the residue, thus obtained, showed on TLC many close spots with a heavy streaking. With potassium ferricyanide–$FeCl_3$ spray reagent their phenolic nature was indicated. Further attempts at purification by chromatography on Si gel and polyamide led to fractions with markedly enhanced activity but no pure substance could be isolated. No cardioactive component could be detected in this species.

The benzene-soluble fraction of the extract was partitioned between hexane and 90% MeOH. The latter fraction was identical with the butanol-soluble material. The hexane fraction was chromatographed over silica gel and neutral alumina which led to the isolation of two pure substances, A and B.

Substance A, designated as mallotin, $C_{32}H_{54}O$ (M^+ 454), showed diagnostic colour reactions of an unsaturated triterpenoid. The IR spectrum of mallotin showed absorption for hydroxyl (3400), methylene group (1645, 895) and a trisubstituted double bond (821 cm^{-1}). Its NMR spectrum exhibited signals for eight C-methyls, a vinylic methyl, a CHOH (1H at 1·49, D_2O exchangeable and 1H at 3·45), a methylene group (4·75 ppm) and an olefinic proton multiplet (5·40 ppm).

The presence of a secondary hydroxyl group was supported by the formation of a monoacetyl derivative, $C_{34}H_{56}O_2$ (M^+ 496). The IR spectrum of acetyl mallotin did not exhibit absorption for hydroxyl group, its NMR spectrum showed a singlet at 2·05 ppm due to the acetoxy group and the carbinol proton appeared as a multiplet at 4·72 ppm. When mallotin was treated with Jone's reagent, a keto derivative, $C_{32}H_{52}O$ (M^+ 452) was obtained which showed a strong absorption at 1712 cm^{-1} (carbonyl in a six membered ring).

The hydrogenation of mallotin in the presence of Adam's catalyst yielded the dihydro derivative, $C_{32}H_{56}O$ (M^+ 456). The IR spectrum of dihydromallotin, was devoid of a methylene band but the absorption corresponding to the trisubstituted double bond was still present. In the NMR spectrum, the signals corresponding to a methylene and a vinylic methyl were replaced by signals due

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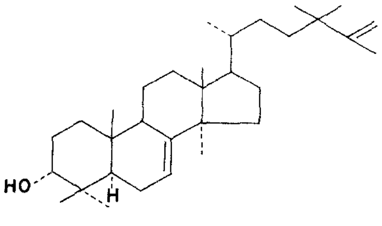
to two secondary methyls which confirmed the presence of a isopropenyl group in mallotin.

The dihydromallotin could not be hydrogenated to a tetrahydro derivative even at 80° and under 500 lb pressure indicating the hindred nature of the trisubstituted double bond. However, dihydromallotin when reacted with *m*-chlorperbenzoic acid, formed an epoxide, $C_{32}H_{56}O_2$ (M^+ 472). Its IR spectrum exhibited the characteristic absorption for an epoxide ring (914 cm^{-1}) and the vinylic proton signal in the NMR spectrum was replaced by a one proton multiplet centered at 3·06 ppm.

In view of the above data, it was evident that the molecule contained four rings and was, therefore, a C_{32} tetracyclic triterpenoid. So far, eight C_{32}-triterpenoids are known which possess the lanostane skeleton with a 10 carbon sidechain [10–13]. In six of them the additional two carbons are present as methyls at C-24 while in the other two substances, one extra methyl is at C-24 and the other is at C-25. By analogy with the above substances and because of the presence of an isopropenyl group, mallotin was considered to possess the 24,24-dimethyl lanostane skeleton. This was supported by the almost identical mass spectral fragmentation pattern of mallotinone compared with that of 24,24-dimethyl lanosta-9(11), 25-dien-3-one*. Both substances showed prominent MS peaks at *m/e* 452 (M^+), 437, 423, 395, 381, 368, 353, 339, 325, 311, 299, 271, 257, 245, 231, 187, 161, 137, 109, 95, 69 and 55.

The position of the methylene group was assigned at C-25 because this would be the only available position in the molecule which could constitute the desired isopropenyl grouping. The choice between the two possible positions Δ^7 or $\Delta^{9(11)}$ for the trisubstituted double bond was made on the basis of the NMR chemical shift of the olefinic proton and the reactivity of the double bond. The chemical shift (5·40 ppm) of the olefinic proton in mallotin differed considerably from that (5·20–5·27 ppm) reported for the $\Delta^{9(11)}$ bond in the known substances mentioned above, thus indicating that it was located at the Δ^7 position in mallotin. This inference was also in accord with its extreme inertness towards hydrogenation [14], whereas the $\Delta^{9(11)}$ bond is susceptible to catalytic hydrogenation [10].

The secondary hydroxyl was assigned to the C-3 position in view of the fact that triterpenes in general carry an oxygen function at this position, and on the basis of the positive Zimmermann test shown by mallotinone. Since the $W_{1/2}$ of the carbinol proton signal was of the order of 5 Hz, it was assigned the equatorial 3β configuration and the hydroxyl would consequently have the 3α configuration which is rather uncommon in triterpenoids. The structure of mallotin is therefore proposed as 24,24-dimethyl lanosta-7,25-dien-3α-ol(I).



(I)

Substance B, mp 137°, $(\alpha)_D$ −38°, $C_{29}H_{50}O$, gave a monoacetate, mp 126°, and was identified as sitosterol.

EXPERIMENTAL

All mps are uncorrected. The NMR spectra were recorded in $CDCl_3$ with TMS as internal standard.

The EtOH extract of the whole plant (ariel parts, 14 kg, voucher specimen is kept in CDRI) was exhaustively macerated with C_6H_6 and the insoluble portion was partitioned between *n*-BuOH and H_2O. The C_6H_6-soluble fraction (200 g) was partitioned between hexane and 90% MeOH, the hexane fraction was decolorized with activated charcoal and the resultant residue (60 g) was chromatographed on Si gel.

The residue from the C_6H_{14}–C_6H_6 (1:2) eluate (6·36 g) was rechromatographed over neutral alumina and crystallized from $CHCl_3$–MeOH as colourless needles, (substance A, 0·50 g). The C_6H_6–MeOH (99:1) eluate crystallized from MeOH as colourless needles, mp 137° (substance B, 0·35 g).

Mallotin (*substance A*). Mp 190°, $(\alpha)_D$-68° (*c*. 1, $CHCl_3$). It gave a violet colour with Ac_2O–H_2SO_4 as well as $SOCl_2$ and a deep yellow colour with tetranitromethane. ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950, 1470 and 1380 (CH_2, Me), 3090, 1645 and 895 ($-C{=}CH_2$), 3042 and 821 ($-C{=}CH$), 1230, 1070, 982 and 940. NMR (60 MHz): δ 0·68, 0·71 (3H each, *s*, 2 × Me), 0·93 (9H, *s*, 3 × Me), 1·03 (6H, *s*, 2 × Me), 1·10 (3H, *s*, Me), 1·70 (3H, *brd s*, vinylic Me), 1·49 (1H, *brd s*, D_2O exchangeable), 3·45 (1H, *m*, $-C\underline{H}OH$), 4·75 (2H, *m*, $-C{=}CH_2$), 5·40 (1H, *m*, $-C{=}CH-$). MS: *m/e* 454 (M^+), 439, 421 ($M - H_2O - Me$), 407, 313, 283, 259, 241 and 189. (Found: C, 84·60; H, 11·60, $C_{32}H_{54}O$ requires: C, 84·60; H, 11·87%).

Mallotin acetate. Mallotin (100 mg) and Ac_2O in C_5H_5N

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* Sample obtained from Dr. W. H. Hui, Hong Kong.

were allowed to stand overnight and then worked up. The residue was crystallised from $CHCl_3$–MeOH, mp 160–2°, 84 mg ν_{max}^{KBr} cm^{-1}: 2907, 1462, 1745 and 1240 (–OAc), 1640 and 895 ($C{=}CH_2$) and 816 (C=CH–). NMR: δ 0·68, 0·75, 0·86, 0·98 (3H each, *s*, 4 × Me), 1·00 (3H, *d*, *J* 7 Hz, –CH–C$\underline{H}_3$), 1·03 (6H, *s*, 2 × Me), 1·10 (3H, *s*, Me), 1·72 (3H, *brd s*, vinylic Me), 2·05 (3H, *s*, –OCOMe), 4·72 (3H, *m*, $C{=}CH_2$ and –CH–O–Ac), 5·35 (1H, *m*, –C=CH–).

Dihydromallotin. Mallotin (50 mg) in EtOAc was shaken in a H_2 atmosphere in the presence of PtO_2 for 2 hr. The residue crystallised from C_6H_6–MeOH, mp 162°, (35 mg). ν_{max}^{KBr} cm^{-1}: 3344 (OH), 2907, 1462 and 1372 (–CH=C–). NMR: δ 0·66, 0·70 (3H each, *s*, 2 × Me), 0·77 (3H, *s*, Me), 0·80 (6H, *d*, 2 × sec. Me), 0·86 (3H, *s*, Me), 0·93 (9H, *s*, 3 × Me), 1·09 (3H, *s*, Me), 3·43 (1H, *m*, –C$\underline{H}$OH), 5·36 (1H, *m*, –C=CH–).

Dihydromallotin epoxide. Dihydromallotin (80 mg) in $CHCl_3$ was reacted with *m*-chloroperbenzoic acid (90 mg) for 48 hr. After usual work up, the residue, showing 1 major and 4 minor spots on TLC, was chromatographed on Si gel. The C_6H_6–$CHCl_3$ (2:1) eluate crystallised from MeOH, mp 151–5°, 30 mg, R_f 0·34 in C_6H_6–$CHCl_3$ (1:1). ν_{max}^{KBr} cm^{-1}: 3401 (OH), 2907, 1462, 1381, 1071, 914 (epoxide). NMR: δ 0·76, 0·84 (6H each, *s*, 4 × Me), 0·90 (12H, *s*, 4 × Me), 1·10 (6H, *s*, 2 × Me), 3·06 (1H, *m*, H on epoxide), 3·38 (1H, *m*, –C$\underline{H}$OH). MS: *m/e* 472 (M^+), 458, 457, 443, 439, 331, 318, 313, 249, 234 and 175 (found: C, 80·95; H, 12·20, $C_{32}H_{56}O_2$ requires: C, 81·21; H, 11·95%.

Mallotinone. Mallotin (50 mg) was reacted with 8N chromic acid and the derivative was crystallized from $CHCl_3$–MeOH, mp 154–5° (35 mg). ν_{max}^{KBr} cm^{-1}: 2907, 1441, 1372, 1712 (carbonyl). 898 ($C{=}CH_2$), 817. NMR: δ 0·66, 0·78 (3H each, *s*, 2 × Me), 1·01 (6H, *s*, 2 × Me), 1·05, 1·13 (3H each, *s*, 2 × Me), 1·30 (6H, *s*, 2 × Me), 4·70 (2H, *m*, –$C{=}CH_2$), 5·40 (1H, *m*, –C=CH–).

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