Phytochemistry, 1967, Vol. 6, pp. 735 to 739. Pergamon Press Ltd. Printed in England

STRUCTURE OF MURICATIN

S. N. KHANNA and P. C. GUPTA

Chemical Laboratories, University of Allahabad, Allahabad, India

(*Received* 9 *August* 1966)

Abstract—4-Hydroxy-14-*O*-biglucoside ethyl stearate and 14-*O*-biglucoside-γ-stearolactone structures were assigned to muricatin A and B respectively.[4] However, the present studies show that muricatin A is not a homogeneous compound. Muricatin B has been shown to consist of a 4-*O*-L-rhamnopyranosyl-L-rhamnopyranose moiety attached glycosidically to (+)-11-hydroxyhexadecanoic acid.

VALETTE and Liber[1] reported that various plants of the family Convolvulaceae, contain glycosides which have a bactericidal effect. Recently[2] it has been noticed that convolvulaceous glycosides bear close resemblance to some of the bacterial metabolites in their general make-up. Some of these bacterial metabolites, i.e. glucoustilagic acid,[3] possess antibiotic properties. Therefore, it seemed advisable to investigate the seeds of *Ipomoea muricata* (Convolvulaceae) for its active constituents.

Misra and Tewari[4] reported the isolation of several constituents from *I. muricata* including muricatin A (4-hydroxy-14-*O*-biglucoside-ethyl stearate) and muricatin B (14-*O*-biglucoside-γ-stearolactone). Some facts, like the homogeneity of muricatin A, the interconversion of muricatin A and B, the nature of sugars were not elucidated. Muricatin B has a marked physiological activity,[5] and so a closer study of this active constituent was undertaken.

Muricatin A was isolated by a similar method to that described[4] and found to be identical in its physical constants to the compound described by previous workers. The i.r. spectrum in chloroform showed very strong hydrogen bonded hydroxyl absorption (2·9 μ), a moderate ester or other carbonyl band (5·8 μ), a very strong and broad absorption (9·2 μ) suggesting ether linkages, and moderate absorption (11·2 μ) indicating the possibility of β-glycosidic linkages. Examination by TLC suggested the presence of at least three components, but repeated crystallization from different solvents failed to yield a homogeneous product. Attempts to purify the compound on silica gel column were also unsuccessful. Hydrolysis of the mixture with ethanolic potassium hydroxide gave muricatin B along with a mixture of acids and glucose. The free acids were separated into steam-volatile and non-volatile portions. The steam-volatile fraction was identified as *n*-caproic acid and the non-volatile fraction contained palmitic and stearic acids.

Elementary analysis of muricatin B indicated the empirical composition, $C_{28}H_{52}O_{11}$. Periodate oxidation showed that approximately 3 moles of periodate were consumed per equivalent of the glycoside, calculated on the basis of the above formula. The i.r. spectrum in KBr showed absorption at 2·95 μ, 5·83 μ, 9·3 μ and 11·2 μ. Muricatin B had a neutralization equivalent 545, and acid hydrolysis yielded 2 moles of L-rhamnose and 1 mole of fatty

[1] G. VALETTE and A. LIBER, *Compt. Rend. Soc. Biol.* **128**, 362 (1938).
[2] C. R. SMITH, L. H. NIECE, H. F. ZOBEL and I. A. WOLFF, **3**, 289 (1964).
[3] R. U. LEMIEUX, *Can. J. Chem.* **29**, 415 (1951).
[4] A. L. MISRA and J. D. TEWARI, *J. Indian Chem. Soc.* **30**, 391 (1953).
[5] S. N. KHANNA and P. C. GUPTA, Unpublished data.

acid. Crude muricatin B, on hydrolysis, showed the presence of a small amount of glucose, but the carefully recrystallized product from dry acetone gave only L-rhamnose. It seems most likely, therefore, that the previous report that glucose was the sugar component of the glycoside is incorrect.

The aglycone was converted to a crystalline methyl derivative which, on saponification and subsequent acidification, yielded an acid, $C_{16}H_{32}O_3$. The i.r. spectra of this acid in KBr showed that it is a saturated aliphatic acid, and the i.r. spectrum of the methyl ester indicated the presence of an hydroxyl group (2·8 μ) which was confirmed by the preparation of mono-phenylurethane derivative. Oxidation of the methyl ester with chromium trioxide and acetic acid yielded pentanoic, hexanoic, sebacic acids and the half-ester of undecanedioic acid. These fragments show that the hydroxyl group is located at C_{11} in a normal C_{16} carbon chain. The melting points of the free acid and its methyl ester, together with optical rotation and oxidation studies, indicate that aglycone is (+)-11-hydroxyhexadecanoic acid, probably identical with jalapinolic acid. The constants reported by Davies and Adams[6] also agree with those recorded by the authors.

	This work	Davies and Adams[6]	Misra and Tewari[4]
Acid	65–66°	68–69°	56–57° (waxy)
Ester	43–44° $[\alpha]_D^{25}+0·7°$	41–42° $[\alpha]_D^{25}+0·8°$	44–45°

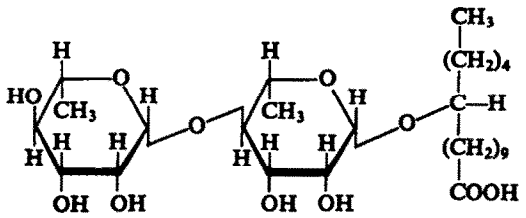
Neither i.r. nor colour reaction[7] indicated the presence of a lactonic group in aglycone, as reported earlier.[6] Nor were any crystalline derivatives found to justify the suggested dihydroxy nature of the aglycone.[6] Possibly the other Indian authors were dealing with an impure product. No C_4 dicarboxylic acid was found on oxidation of methyl ester of aglycone, which should have been produced if the dihydroxyoctanoic acid structure[6] were correct. The low melting points of the aglycone and its ester (56–57°, 44–45°) also do not justify the dihydroxystearic acid structure.

The present studies showed that muricatin A is not a homogeneous product. On alkaline hydrolysis it yields, apart from muricatin B, a mixture of fatty acids and glucose. The possibility of lower molecular weight alcohols also being formed cannot be excluded. In view of the above facts, the structure of muricatin A as an ethyl ester does not seem convincing. The production of free glucose on alkaline hydrolysis showed that the hydroxyl groups in the latter were most probably esterified by fatty acids (*n*-caproic, palmitic and stearic). But other possibilities, such as presence of free acids, esterification by lower alcohols or esterification of the free hydroxyls of rhamnose, cannot be excluded.

The difference between the molecular formula for muricatin B and its aglycone shows that it contains 2 moles of sugar. The observed consumption of 3 moles of periodate per mole of muricatin B would indicate that the two L-rhamnose units were joined either by a $1 \rightarrow 4$ linkage as shown in the formula (I) or by a $1 \rightarrow 2$ linkage which is rarely encountered in higher plants. In view of the greater stability of pyranose compared to furanose form,[8] the L-rhamnose is probably as shown in I. The absorption maximum at 11·2 μ in the i.r.

[6] L. A. DAVIES and R. ADAMS, *J. Am. Chem. Soc.* **50**, 1749 (1928).
[7] FEIGEL, *Spot Test in Organic Analysis*, p. 287. Elsevier, Amsterdam (1956).
[8] F. SHAFIZADEH, *Advances in Carbohydrate Chemistry*, Vol. 13, p. 9. Academic Press, New York (1958).

spectra of muricatin B indicates an axial hydrogen[9] at C-1 (β-D-glycosidic linkages). It is noteworthy in this respect that the i.r. absorption spectrum of muricatin B in the region 7·5–12·0 μ was nearly identical to the spectrum of glucoustilic acid.[3] This indicates that the sugar–fatty acid linkages are β. The previous work of Tewari and Misra[4] also showed the probability of β-linkages by emulsion hydrolysis. The negative rotation, $[\alpha]_D^{25} -44{\cdot}5°$, of the glycoside indicates a β-configuration for the rhamnose–fatty acid linkage, as well as that of rhamnose to rhamnose. The free hydroxyl present at C_{11} in the aglycone is involved in the linkage to the sugar (I).



(I)

Muricatin B is thus a 4-*O*-L-rhamnopyranosyl-L-rhamnopyranose moiety attached glycosidically to (+)-11-hydroxyhexadecanoic acid. The presence of both free (+) and glycosidically bound (+)-11-hydroxyhexadecanoic acid have been reported several times, but its dirhamnoside has not been reported previously.

EXPERIMENTAL

Melting points are uncorrected. The i.r. spectra were taken on Perkin-Elmer Infracord. Paper-chromatographic analyses were carried out at room temperature.

Isolation of Muricatin A

The muricatin A was isolated from the alcoholic extract of the seeds as described by Misra and Tewari.[4] Thin-layer chromatography was carried out on silica-gel plates, using the solvent system, chloroform:methanol (9:1) and 50% sulphuric acid as spray. The presence of three components were indicated. 1·0 g of muricatin was adsorbed over a column of silica-gel and the elution was carried out by chloroform:methanol (9:1). 25 ml of elutes were collected and examined over silica-gel plates which showed incomplete resolution of the components. Infrared spectrum in chloroform showed absorption at 2·9 μ (strong and broad); 5·8 μ (strong); 9·2 μ (strong and broad) and 11·2 μ (weak).

Alkaline Hydrolysis of Muricatin A

The hydrolysis of muricatin A (40·0 g) was carried out with 500 ml of N/2 ethanolic NaOH at 100° for 6 hr. After hydrolysis the solvent was distilled off, and the residue taken up in water and extracted several times with ether to remove unsaponifiable matter. The aqueous layer was acidified with dilute H_2SO_4 and shaken with ether. The mixture separated into three layers: (*a*) upper ethereal layer, (*b*) a middle dark viscous layer and (*c*) the lower aqueous layer. The three layers were separated and examined.

[9] S. A. BARKER, E. J. BOURNE, M. STACEY and D. H. WHIFFEN, *J. Chem. Soc.* 177 (1954).

(*a*) *Examination of ethereal layer.* The combined ether extracts were evaporated, when a semi-solid mass was obtained (10·2 g). The steam-volatile acid was separated and extracted from the distillate with ether (1·2 g). It was optically inactive, and paper chromatography by the method of Kennedy and Barker[10] (solvent system, 100 ml 95% ethanol, 1 ml conc. ammonium hydroxide) showed the presence of a single component, R_f 0·69 (*n*-caproic acid, R_f 0·68). The acid gave an amide, m.p. and m.m.p. 97–98°. The non-volatile portion was extracted with ether, the extract dried, concentrated and treated with diazomethane. The esters so obtained were subjected to fractional distillation under reduced pressure (4 mm). Two fractions, one boiling at 170° and the other at 177–180°, were collected. The fatty acids were recovered by saponification and repeatedly crystallized from acetone. They were shown to be palmitic acid, m.p. 62–63°, and stearic acid, 67–68°, confirmed by m.m.p. point determination.

(*b*) *Examination of middle oily layer.* It was dried to a viscous dark gum which was dissolved in dry acetone and refluxed with charcoal. On removal of the solvent, an almost colourless mass was obtained which was carefully crystallized from dry acetone, m.p. 108–109°. It was identical to the muricatin B, reported by Misra and Tewari,[4] in all its physical constants.

(*c*) *Examination of aqueous layer.* The aqueous layer was neutralized with $BaCO_3$ and filtered. The filtrate was concentrated to a syrup, which was examined by paper chromatography (solvent, *n*-butanol:ethanol:water (40:10:50)), aniline hydrogen phthalate was used as spray reagent, R_f 0·085 (glucose 0·09). The compound is crystallized from aq. methanol, m.p. and m.m.p. 145°; Osazone, m.p. and m.m.p. 205–206°.

Muricatin B

The i.r. spectrum in KBr showed absorption at 2·95 μ, 5·83 μ, 9·3 μ, and 11·2 μ. There was no selective absorption in the u.v. region. Optical rotation in ethanol, $[\alpha]_D^{25} = -44{\cdot}5°$. (Found: C, 59·5; H, 9·4; neutral equivalent, 545. $C_{28}H_{52}O_{11}$ required: C, 59·6; H, 9·3; neutral equivalent, 564.)

Periodate oxidation of muricatin B. To the glycoside (0·545 g), in water (25 ml), was added sodium metaperiodate (25 ml of 0·25 M) and the volume was made up to 100 ml with water. 5 ml aliquots were titrated against thiosulphate (0·01 N) and showed the consumption of 3·2 moles of periodate per mole of glycoside.

Acid hydrolysis of muricatin B. 10·0 g of muricatin B was dissolved in 300 ml of 2% H_2SO_4 heated at 100° for 5 hr. After cooling, the contents were extracted with ether, and the washed ethereal layer dried (Na_2SO_4) giving a waxy aglycone (4·61 g). The aqueous solution was neutralized ($BaCO_3$), filtered, and evaporated to a syrup (4·90 g). Paper chromatography of the syrup showed the presence of single component, R_f 0·29 (L-rhamnose, R_f 0·30). The sugar was crystallized from aqueous methanol, m.p. and m.m.p. 98–99°; Osazone, m.p. and m.m.p. 179–180°.

Characterization of Aglycone

The aglycone was refluxed for 12 hr with 20 ml of methanol saturated with dry HCl giving the ester which was crystallized from light petroleum (40–60°), m.p. 43–44°, $[\alpha]_D^{25}$ + 0·7°. Its i.r. in KBr showed maxima at 2·8 μ (hydroxyl group) and 5·75 μ (ester). 1·0 g of the ester was boiled with ethanolic NaOH for 6 hr. The solvent was removed and the sodium

[10] E. P. KENNEDY and H. A. BARKER, *Anal. Chem.* **23**, 1033 (1952).

salt taken up in water, and the acid liberated on acidification (H_2SO_4) and extracted with ether. The combined ether extracts were dried (Na_2SO_4) giving a product which was first crystallized from light petroleum (60–80°) and then from aqueous acetone, m.p. 65–66°. The i.r. in KBr showed absorption at 3·05 μ, 3·5 μ, 3·55 μ, 5·85 μ and 6·8 μ. (Found: C, 70·4; H, 11·6%; mol. wt. (Rast), 258, 262; neutralization equivalent, 268. Calc. for $C_{16}H_{32}O_3$: C, 70·56; H, 11·8%; mol. wt., 272; neutralization equivalent, 272.) 100 mg of the ester and 150 mg of phenylisocyanate with a drop of pyridine was heated at 100° for 15 min giving a urethane, m.p. 47–48°. (Found: C, 70·5; H, 9·46; N, 3·40%; mol. wt. (Rast), 396. Calc. for $C_{23}H_{37}O_4N$: C, 71·1; H, 9·62; N, 3·45; mol. wt., 405.)

2·0 g of the methyl ester was oxidized with chromium trioxide–acetic acid as described by Meakins and Swindells.[11] The reaction mixture was diluted with ice-water, extracted with ether, and the extracts extracted with 5% K_2CO_3. The carbonate extracts were acidified (HCl) and extracted with ether. The dried (Na_2SO_4) ether extracts were evaporated cautiously so as to retain volatile acids. The mixture of acids was then separated into steam-volatile and non-volatile portions. The steam distillate on ether extraction and subsequent evaporation of the solvent gave an oily liquid. Paper chromatography[10] showed the presence of two acids, R_f 0·62 and 0·69 (R_f for valeric, 0·60, and for caproic, 0·68). The non-volatile acidic portion was extracted with ether, the ether was distilled off, and the product (1·10 g) was dissolved in 10 ml of ethyl acetate giving a solid which on recrystallization had m.p. 129–131°. (Found: C, 59·2; H, 8·89%; mol. wt. (Rast), 196. Calc. for $C_{10}H_{18}O_4$: C, 59·40; H, 8·91% mol. wt., 202.) It was confirmed as sebacic acid by m.p. and m.m.p.; amide, m.p. and m.m.p. 196–197°.

The mother liquor, after the separation of sebacic acid, was saponified with excess of ethanolic KOH. The alcohol was distilled off and the product acidified (dil. H_2SO_4) and extracted with ether. The ether extract was concentrated and examined on paper chromatograms, by the method of Isherwood and Hanes[12] for dicarboxylic acids (solvent, n-propanol: conc. aqueous ammonia, 60:40), bromophenol blue was used as spraying reagent. Two spots were observed, R_f 0·63 and 0·69 (sebacic acid, R_f 0·65). The slow-moving substance (R_f 0·63) was confirmed to be sebacic acid by chromatography with an authentic sample.

[11] G. D. Meakins and R. Swindells, *J. Chem. Soc.* 1044 (1959).
[12] F. A. Isherwood and C. S. Hanes, *Biochem. J.* **48**, 824 (1953).