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A NEW SAPONIN FROM SEEDS OF *AMOORA ROHITUKA*

S. K. BHATT, V. K. SAXENA and S. S. NIGAM*

Department of Chemistry, University of Saugar, Sagar (M.P.) 47003, India

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Key Word Index—*Amoora rohituka*; Meliaceae; saponin; stigmasta-5,24(28)-dien-3β-*O*-β-D-glucopyranosyl-*O*-α-L-rhamnopyranoside.

Abstract—Phytochemical examination of the seeds of *Amoora rohituka* resulted in the isolation and identification of a new saponin, stigmasta-5,24(28)-dien-3β-*O*-β-D-glucopyranosyl-*O*-α-L-rhamnopyranoside.

Amoora rohituka (Meliaceae) is used as a medicinal plant [1,2]. A phytochemical examination of the seed was undertaken by us because very little work on this species has been reported [3,4].

The saponin, mp 65–66°, gave a copious lather when shaken with water and it also haemolysed red blood cells. Hydrolysis of the saponin with 7% H_2SO_4–EtOH afforded genin and sugars which were identified as D-glucose and L-rhamnose (by co-PC). The genin was crystallized from $CHCl_3$–MeOH as colourless needles, mp 121–124°, $C_{29}H_{48}O$ (M^+ at *m/z* 412), $[\alpha]_D^{20}$ −37° (in $CHCl_3$). It formed an acetyl derivative [5,6] (Ac_2O/pyridine), mp 117–118° (M^+ at *m/z* 454. (Found: C, 81.87; H, 11.1; OMe, 9.44%). The benzoate derivative had mp 118–120°, (M^+ at *m/z* 516). (Found: C, 83.70; H, 10.04; $C_{36}H_{52}O_2$ required: C, 82.72; H, 10.7%). It also formed a digitomide, mp 232–234°, showing a hydroxyl function in the molecule. The saponin did not form an acetate or benzoate derivative indicating the absence of a free hydroxyl function in the saponin.

On Oppenauer oxidation the genin afforded stigmasta-4,24(28)-diene-3-one (α,β-unsaturated ketone), mp 93–94°, thus indicating the presence of a Δ^5-3β-OH grouping in the genin [7]. The genin on ozonolysis yielded acetaldehyde which was converted to the acetaldehyde-*p*-nitrophenyl hydrazone, mp 126–128° (TLC and mmp), which located the second double bond at the $\Delta^{24(28)}$ position [8]. Therefore the genin was assigned the structure stigmasta-5,24(28)-diene-3β-ol, which was confirmed by mmp and co-TLC with an authentic sample [9].

The periodate oxidation of the saponin consumed 3.2 mol of periodate with the liberation of 1.3 mol of formic acid per mol of the saponin [10] which indicated the presence of a disaccharide having both the units in the pyranose form. The saponin on partial hydrolysis (with 2% H_2SO_4) yielded glucose which suggested that it occupied the terminal position in the sugar moiety and rhamnose was linked with the genin at position C-3. The permethylated [11] saponin on acid hydrolysis yielded 2,3-di-*O*-methyl-L-rhamnose (by co-TLC with authentic material) and 2,3,4,6-tetra-*O*-methyl glucose (mmp and co-chromatography with an authentic sample). This indicated that the glucopyranose unit was joined to the rhamnopyranose unit by a 1 → 4 linkage. The enzymatic hydrolysis [12] of the saponin showed a β-linkage between the two sugars with an α-linkage between the aglycone and the rhamnose. Therefore the new saponin was assigned the structure stigmasta-5,24(28)-dien-3β-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside.

*Senior investigator.

EXPERIMENTAL

Air-dried seeds of *Amoora rohituka* Roxb. (2 kg) were obtained from James Td. Co., Dehradun (U.P.) and identified by the Botany Department, University of Saugar. The seeds were extracted by refluxing with EtOH (95%) for 15 days. The extract (2.5 l.) was concd under red. pres. (100 ml) and poured into distilled H_2O (500 ml) with continuous stirring. The water-soluble portion on extraction with C_6H_6 gave the saponin (1.2 g). It was purified on a column of neutral alumina, crystallized as white needles with $CHCl_3$–MeOH, mp 65–66°. The homogenicity of the saponin was checked by PC in *n*-BuOH–HOAc–H_2O (4:1:5), R_f 0.40. IR ν_{max} cm^{-1}: 3420 (OH group), 2952, 1050, 1575 and 800 (strong) ($\Delta^{24(28)}$ ethylidene sterol), 1475, 1390 and 955 (*iso*-propyl group). ^1H NMR ($CDCl_3$): δ 0.81 (*s*, H-19), 0.99 (fused *d*, H-21, H-26 and H-27), 102 (*s*, H-18), 1.55 (*d*, H-29), 2.81 (*septet*, H-25), 5.02 (*q*, H-28) and 4.7 (*br m*, H-6).

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A BROMOPHENOL IN THE RED ALGA *HALOPITYS INCURVUS*

GUILLAUME DE NANTEUIL and PIERRE MASTAGLI

Ecole Supérieure de Chimie Organique et Minérale, 12, rue Cassette, 75006 Paris, France

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Key Word Index—*Halopitys incurvus*; Rhodomclaceae; bromophenol; 2,6-dibromo-3,5-dihydroxyphenylacetic acid.

Abstract—A new dibromophenol has been isolated from the acidified ethanolic extracts of the red alga *Halopitys incurvus*, and is shown to be 2,6-dibromo-3,5-dihydroxyphenylacetic acid, probably derived from a disulfate dipotassium salt.

INTRODUCTION

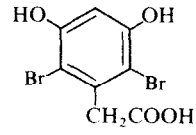
The existence of brominated phenolic compounds in the extracts of *Halopitys incurvus* (Hud.) Batt. has been known since Augier and Mastagli [1] described a sulfonic acid, dipotassium salt. Later Chantraine [2] and Glombitza [3, 4] isolated various phenolic acids and benzyl alcohols, identified after various extraction procedures.

RESULTS AND DISCUSSION

After acid hydrolysis, the aqueous EtOH extracts of *Halopitys incurvus* (5 kg dry alga) yielded 2.5 g of a compound which gave a positive ferric chloride test. A bromine analysis [5] showed the presence of two atoms per molecule (found: Br, 49.02. $C_8H_6Br_2O_4$ requires: Br, 49.08%). IR, ^{1}H NMR, ^{13}C NMR broad band and off resonance decoupling spectra showed the presence of single aromatic hydrogen and a phenylacetic group. Moreover, the broad band spectrum presented only four peaks for the aromatic ring, which implied a structure similar to 4,6-dibromoresorcinol.

To determine the phenylacetic group, we synthesized the 4,6-dibromoresorcinol [6]; comparison of its ^{1}H NMR spectrum with that of the algal compound showed that the phenylacetic group is most probably situated between the two bromine atoms. Hence the new compound has structure **1**.

When the aq. EtOH extracts were saturated with absolute alcohol, several mg of a very hygroscopic product crystallized, giving a purple colour with 2% aqueous $FeCl_3$ and decomposing at a temperature higher than 250°. The IR spectrum showed the presence of a phenylacetic group. Analysis also gave two bromine atoms per molecule (found: Br, 27.12. $C_8H_3Br_2O_{10}S_2K_2Na$ requires: Br, 27.39%). The lability of the product permitted only a qualitative determination of sulfate, potassium and lesser amounts of sodium. We therefore assume that this compound is the disulfate dipotassium salt of the diphenol **1**, present as the sodium salt [1, 7, 8]. During extraction, approximately 2 g needles of (2-glyceric acid sodium salt) α-D-mannopyranoside [9] crystallized and were identified by their IR, ^{1}H and ^{13}C NMR spectra; they decomposed at above 250°.



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EXPERIMENTAL

Halopitys incurvus was collected in September at low tide in the Rade de Brest (Brittany). Immediately after harvest, the alga was extracted with cold EtOH (35 l) for 2 weeks, then concd to give 3 l. of a dark brown soln.

Hydrolysed compound. This soln was hydrolysed with dil. HCl (10%) and heated on a steam bath for 15 min. After cooling and filtration, the resulting soln was extracted with EtOAc, and the yellow organic extract was washed with water, then dried. The residue after evapn was chromatographed on a column of 100 g Si gel (Merck 70–230 mesh), using $CHCl_3$–MeOH (9:1) as eluant.