strong peaks at *m/e* 366 (deacetylaglycone) and 69 ($C_5H_9^+$). Compound F VII must therefore be *dideacetylfusicoccin* (IV).

Dideacetylfusicoccin was prepared on treatment of fusicoccin (200 mg) in methanol (4 ml) with 0.1 *N* NaOH in methanol (4 ml). After 30 min at room temperature, the solution was neutralized with HCl, the methanol removed in vacuo and the residue extracted with chloroform. The chloroform solution, washed several times with water, was evaporated and the residue crystallized from dry ethyl acetate to give a solid with mp 183–187°; if the crude compound was heated only briefly with ethyl acetate, or if the solvent was not dry, the crystals had mp 116–117° (microanalysis showed a content of 0.5 moles of water per mole of compound). $[\alpha]_D^{25} + 9.5$ ($c = 0.76$ in ethanol). Rf in 4 solvent systems, IR-, NMR- and mass spectra were identical with those of compound F VII. Calc. for $C_{32}H_{52}O_{10}$: C, 64.43; H, 8.77. Found: C, 64.20; H, 8.84.

The structures of compounds F IV and F VII therefore appear to be firmly established, but further work is required to obtain full elucidation of that of F III (isofusicoccin). It is quite possible that the formation of isofusicoccin from fusicoccin only involves a reversible rearrangement concerning the acetyl group present in the glucose moiety; it was in fact observed that on brief treatment at room temperature with 0.2 *M* sodium bicarbonate, isofusicoccin yields not only monodeacetylfusicoccin but also some fusicoccin.

While isofusicoccin is nearly as phytotoxic as fusicoccin in the assay with tomato plants, monodeacetylfusicoccin and dideacetylfusicoccin are respectively 12 and 100 times less active[8,9].

Riassunto. Dai brodi di coltura di *Fusicoccum amygdali* Del. sono stati isolati un isomero della fusicoccina (isofusicoccina), una monodeacetilfusicoccina, in cui manca l'acetile sul residuo del glucosio, e la dideacetilfusicoccina.

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Chitin in the Cephalochordata, *Branchisotoma floridae*

Although chitin has been reported in all the major invertebrate phyla except in the Protozoa and Echinodermata, it seems to be totally absent in the Chordata[1–5]. During the course of an investigation of the skeletal tissues of protochordates, we detected chitin in the gill bars of the cephalochordate, *Branchisotoma floridae* (Figure 1).

Material and methods. Gill bars were isolated from surrounding tissues by a modification of the procedure outlined by Phillis[6]. Whole thoraces of *B. floridae* were macerated in a waring blender with an equal volume of 5% KOH solution for 24 h. The creamy suspension was passed through graded screens with openings of 1.165, 0.589, 0.295 and 0.147 mm with the aid of suction. Standard Tyler screens, customarily used for geological investigations, were fitted to a Buchner funnel with the aid of masking tape. The suspension after this filtration consisted of gill bars, fine tissue debris and dissolved constituents. Centrifugation of the suspension at approximately 1000 rev/min for 15 min isolated the gill bars, and they were washed with 12 *N* HCl to free them of all soluble materials. Differential centrifugation for periods of 15 min in a small international clinical centrifuge completed the purification procedure. The gill bars were then repeatedly washed with water and boiling methanol until they were no longer positive to histochemical tests for protein and lipid. The gill bars thus obtained were treated as required for demonstrating the presence of chitin.

Results. Gill bars withstood treatment for 15 min in saturated aqueous KOH solution at 100 °C. After washing in distilled water, the alkali-treated bars were coloured brown by iodine in KI solution, becoming violet when this was replaced by dilute H_2SO_4 (Figure 2)[7]. Alkali-treated bars were soluble in mineral and acetic acids. Under these conditions, both cellulose and chitin are relatively stable in alkali, but the above colour reaction and solubility in acids are properties shown only by chitin.

Chitin, a polymer of 2-acetamido-2-deoxy-α-D-glucopyranose (N-acetyl-D-glucosamine), yields D-glucosamine on acid hydrolysis and N-acetyl-D-glucosamine on enzymic degradation[8]. The theoretical value of nitrogen for purified chitin is 6.9%[2]. The gill bars in the specimens that we examined displayed these reactions.

The materials were hydrolyzed in sealed tubes with 6 *N* HCl at 160 °C for 6 h. After drying the hydrolysate over P_2O_5 and KOH, the residue was taken up in water and run on a partition chromatogram against D-glucose and D-glucosamine hydrochloride, using 6 different solvent mixtures. The chromatograms were sprayed with aniline hydrogen phthalate, silver nitrate, or the Elson and Morgan reagents. A substance behaving like glucosamine (or galactosamine) was present in large amounts, but no glucose was detected. On conversion to the corresponding pentose by the Stoffyn and Jeanloz procedure B[9], the

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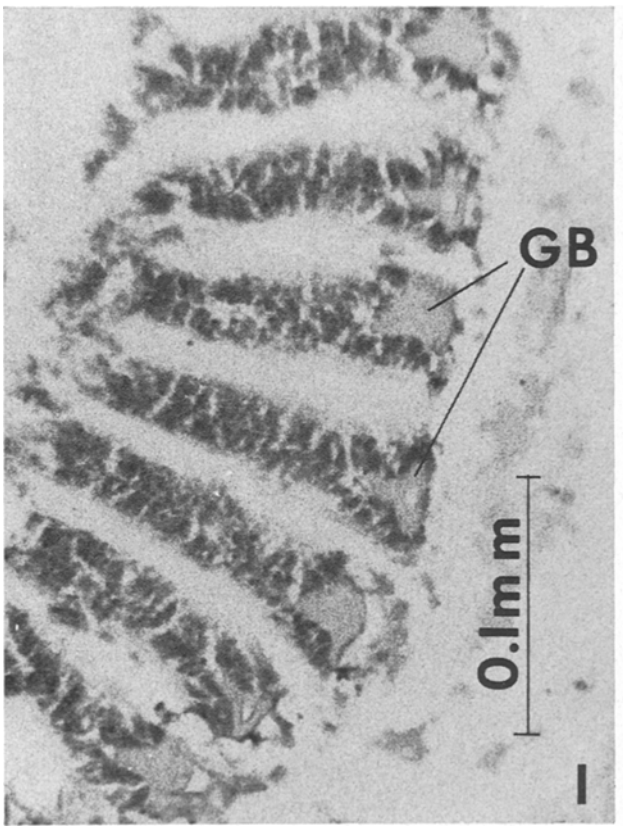

Fig. 1. Transverse section through the gill bars of *B. floridae*. GB, gill bars.

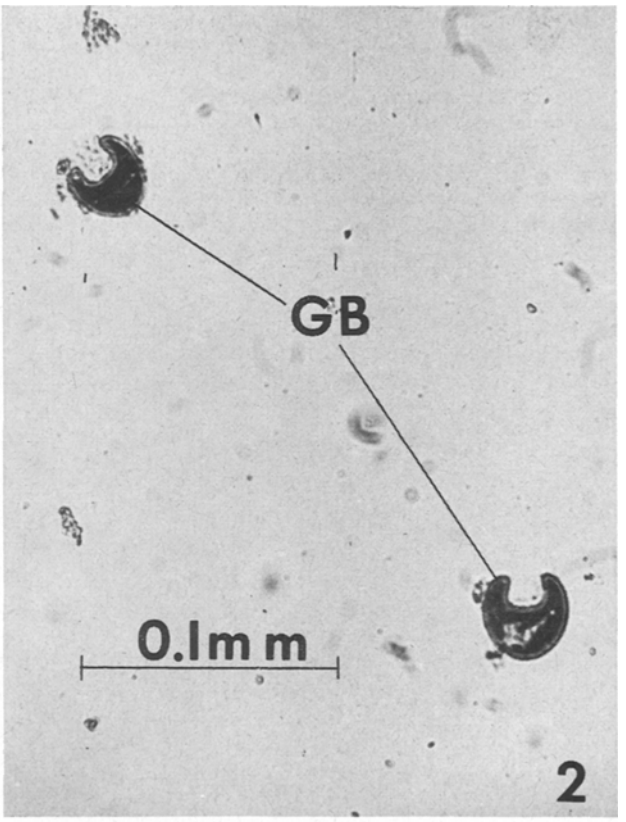

Fig. 2. Isolated gill bars of *B. floridae* after treatment with iodine/sulphuric acid.

acid hydrolysate yielded arabinose, confirming the presence of glucosamine (as opposed to galactosamine).

Another sample of gill bars was incubated at 37 °C with a chitinase and cellulose preparation from the puff ball, *Lycoperdon pyriforme*[10], and buffered at pH 5.0. In samples that were withdrawn after incubation at 18, 42, and 66 h, concentrated in vacuo and chromatographed in a solvent system of butanol-ethanol-water (4:1:5), N-acetyl-glucosamine was shown to be present with the Elson and Morgan reagent, and no glucose was detectable with aniline hydrogen phthalate.

Furthermore, a quantitative estimation of nitrogen with another portion of the sample, after employing the micro Kjeldhal method of STEYERMARK[11], was 6.6%. This is in close agreement with the theoretical value of purified chitin which is 6.9%.

Discussion. Our report is the first investigation to demonstrate the presence of chitin in the protochordates. The presence of cellulose in the mantle of tunicates (Urochordata) is a well established fact[12]. No test seems to have been made on the enteropneusts (acorn worms, Hemichordata). Though RUDALL[2] testifies that the coenocium of the pterobranch, *Rhabdopleura normani* is non-chitinous, SANNASI[13] observed that the coenocium of *Rhabdopleura* spp. displayed positive reactions to the chitosan test.

The occurence of chitin in the cephalochordates, which is unexpected, is of considerable interest. BARRINGTON[14] has recently reported that the cephalochordates are invertebrates, and that they possess well developed protonephridia in their adult stage. The latter organs are characteristic of the lower invertebrates and are otherwise unknown in either the Chordata or Echinodermata. Also characteristic of invertebrates is the bottom-dwelling habit in their adult stage despite their metameric musculature and highly organized nervous system. In view of these facts, the presence of chitin, which is an invertebrate feature, may further substantiate the current doctrine that Amphioxus is more an invertebrate rather than a vertebrate.

Résumé. Présence de chitine constatée pour la première fois dans les septa branchiaux de *Branchisotoma floridae*.

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