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Isolation and Characterization of a Unique Galactoside from Male *Drosophila melanogaster*†

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ABSTRACT: A ninhydrin-positive compound with presumptive hormonal activity, previously considered to be a peptide (Chen, P. S., and Bühler, R. (1970), *J. Insect Physiol. 16,* 615), has been isolated from adult male *Drosophila melanogaster.* Chromatographic analysis of the acid-hydrolyzed material revealed the presence of ethanolamine, phosphorus, galactose, and glycerol. Chemical analysis showed these to be present in equimolar amounts. Based on its phosphorus content, the nonreducing material took up 2 equiv of periodate, and released 1 equiv of formaldehyde. Characterization of the compound as 1-*O*-(4-*O*-(2-aminoethyl phosphate)-β-D-galactopyranosyl)-*x*-glycerol was achieved by gas chromatography–mass spectroscopy and ^1H and ^{31}P NMR using model compounds. In vivo synthesis from labeled precursors is in accord with the proposed structure.

Secretions from the male accessory glands (paragonial glands) of several insects have been examined with a view to identifying compounds having a physiological function in reproduction (cf. reviews by Chen, 1971; de Wilde and de Loof, 1973). The suggestions have been made that paragonial secretions have hormone-like activities, and are involved in the stimulation of egg laying and reduced receptivity of the female toward insemination by other males following an initial copulation. A dimeric protein termed "matrone" has been isolated from males of the yellow fever mosquito *Aedes aegypti* (Fuchs et al., 1969; Fuchs and Hiss, 1970; Hiss and Fuchs, 1972); both protein components were required to elicit monogamy while the α component alone stimulated egg laying. Proteins or protein-like materials having similar effects have been reported from the housefly *Musca domestica* (Nelson et al., 1969; Leopold et al., 1971), fruit fly *Drosophila funebris* (Baumann and Chen, 1973; Baumann et al., 1975), and the grasshopper (*Melanoplus sanquinipes*) (Pickford et al., 1969; Friedel and Gillott, 1976).

The present work is concerned with a major ninhydrin-positive compound from the paragonial glands of adult males of *D. melanogaster* which, from its location on paper chromatography (Chen and Diem, 1961), apparently corresponds to the "sex peptide" reported earlier by Fox (1956a,b) and Fox et al. (1959). This compound was subsequently examined on an amino acid analyzer (Chen and Bühler, 1970), where it eluted as an acidic material between phosphorylserine and glycerophosphorylethanolamine. At that time it was considered to be a peptide in view of the several free amino acids resulting from acid hydrolysis. We now report on the isolation and characterization from this so-called "sex peptide" of an ethanolamine-containing galactoside of unique structure, the earlier conclusions apparently being due to the presence of contaminating peptide(s) cochromatographing with the galactoside in crude preparations.

Materials and Methods

Materials. The wild stock (Sevelen) of *D. melanogaster* was raised on standard diet (corn-sugar-yeast-agar) at 25 °C. Male flies were separated from females about 2 h after adult emergence, and after aging for 8–10 days the males were frozen and stored at −20 °C until required.

Bis(trimethylsilyl)trifluoroacetamide (BSTFA), BSTFA-d_9, and gas chromatographic packings were purchased from Supelco, Inc., Bellefonte, Pa. D-Galactose, D-galactose 6-phosphate, and α-D-galactose 1-phosphate were obtained from Sigma Chemical Co. 1-*O*-(β-D-Galactopyranosyl)-D,L-glycerol was synthesized and kindly donated by Dr. Y. C. Lee (Johns Hopkins University); 1-*O*-(α-D-galactopyranosyl)-D-glycerol, isolated from *Porphyra perforata* (Su and Hassid, 1962), was a generous gift from the late Dr. W. Z. Hassid. Acid phosphatase, phosphodiesterase I, galactose oxidase, and β-galactosidase were purchased from Worthington Biochemical Corp., and the radioisotopes from Amersham-Searle Corp.

Analytical Procedures. Neutral sugar and phosphorus were determined according to Dubois et al. (1956) and Allen (1940), respectively. Ethanolamine was estimated on the amino acid analyzer or, less accurately, after separation by electrophoresis on cellulose TL plates at 25 V/cm at pH 1.9 or 3.7 and elution

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of the ninhydrin-stained zone. Glycerol was determined enzymatically (Wieland, 1970), periodate by the colorimetric procedure of Avigad (1969), and formaldehyde with chromotropic acid (Speck and Forist, 1954).

Physical Procedures. ^{1}H and ^{31}P NMR spectra were obtained with a Varian HA-100 NMR spectrometer. For ^{31}P-^{1}H double resonance, the probe was modified according to Yeh et al. (1973). GLC of alditol acetates was performed as described by Laine et al. (1972) on a column of 3% ECNSS-M on 100/120 mesh Supelcoport.

Mass spectra were obtained on an LKB-9000 gas chromatograph-mass spectrometer at 70 eV. The ion source was maintained at 280 °C and the gas chromatograph was programmed at 10 °C/min to 300 °C using a column of 1% OV-17 on 100 mesh Supelcoport ABW. Samples were trimethylsilylated using bis(trimethysilyl)trifluoroacetamide or its deuterated analogue.

Purification. Fifty-five grams of frozen adult male flies and 100 mL of chilled absolute methanol were homogenized for 10 min at maximum speed in a Büchler homogenizer, the container being surrounded by an ice bath. A further 100 mL of methanol was added and the homogenate filtered through a sintered funnel. The funnel residue was washed with cold 50% (v/v) methanol and the combined filtrates evaporated to dryness on a flash evaporator at 30 °C. The dry residue was taken up in 15 mL of distilled water, and left to stand overnight at 4 °C. A colored precipitate was removed by centrifugation, and the supernatant concentrated to 6.0 mL on the evaporator. At this stage the paragonial substance appeared as a major peak between phosphorylserine and glycerophosphorylethanolamine when run on the amino acid analyzer (Figure 1a).

The extract was then applied to a column (2.6 × 71 cm) of Sephadex G-25 (fine), and eluted with 0.5 M acetic acid at a flow rate of 60 mL/h. After passage of 180 mL, 3.0-mL fractions were collected and 5-μL aliquots from each fraction were spotted onto Whatman No. 1 filter paper for chromatography of ninhydrin-positive compounds using 70% (v/v) aqueous 1-propanol as solvent. The paragonial substance was present in fractions 26–38, which were pooled and evaporated in vacuo.

The residue was dissolved in 1.5 mL of H_2O, acidified with 0.1 mL of concentrated formic acid, and chromatographed on a Dowex 50W-X4 column (2 × 150 cm) with 0.05 M ammonium formate, pH 2.5. After 200 mL of effluent had passed through the column, 3.0-mL fractions were collected and a drop from each fraction tested for the ninhydrin reaction. Fractions 24–40 containing the paragonial substance were combined, concentrated on the evaporator to about 1.5 mL, and desalted on Sephadex G-25 (fine) in 0.5 M acetic acid. A 50-μL sample examined on the amino acid analyzer showed only a single, uncontaminated peak (Figure 1b). Following evaporation, the dried residue was dissolved in 1.0 mL of water and lyophilized. The total yield was 51 mg of dry product (~0.1% based on the frozen fly weight).

Isotopic Labeling of the Paragonial Substance in Vivo. For in vivo labeling of the paragonial material, reproducible results were obtained as follows. Twenty-five male flies were placed in a 25-mL Erlenmeyer flask and kept without food for 1 h. Subsequently, 10 μL of 25% (w/v) sucrose solution containing either 5 μCi of D-[U-^{14}C]galactose (43 mCi/mM, 200 μCi/mL) or [1-^{3}H]ethanolamine (320 mCi/mM, 1 mCi/mL) was pipetted into the flask. A separate drop of water was also added to reduce evaporation of the sugar solution. The flies finished drinking the offered solution within 1–2 h, and control experiments showed that less than 1% of the total radioactivity remained in the flask. The flies were then transferred to culture bottles with standard diet, and used for preparing the methanolic extract at the desired period after feeding. The paragonial substance was then separated on Whatman 3MM filter paper by high-voltage electrophoresis in the first dimension (8% formic acid, pH 1.5, 35 V/cm) followed by chromatography with 70% aqueous 1-propanol in the second dimension (Bauman and Chen, 1973). Subsequent to staining with ninhydrin, the colored paragonial substance zone was cut out from the chromatogram and counted in a liquid scintillation counter (Nuclear Chicago, Mark I) employing 0.8% 2-(4-biphenylyl)-5-(*p-tert*-butylphenyl)-1,3,4-oxadiazole in toluene (efficiency: 75% for [^{14}C]galactose, 7% for [^{3}H]ethanolamine).

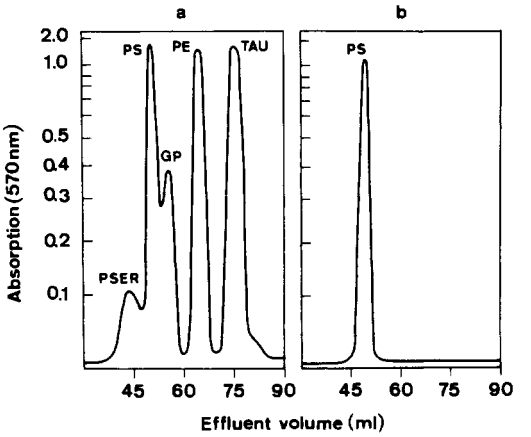


FIGURE 1: Chromatographic profiles of crude (a) and purified (b) paragonial substance on the amino acid analyzer. Abbreviations: PSER, phosphoserine; PS, paragonial substance; GP, glycerophosphorylethanolamine; PE, phosphorylethanolamine; TAU, taurine.

Results

Chemical Analyses. The paragonial substance when hydrolyzed with 6 N hydrochloric acid in an evacuated tube at 110 °C for 18 h yielded only free ethanolamine on the amino acid analyzer, and hence could not be a peptide. From the position of the elution peak of the paragonial substance on the analyzer, it was surmised that the compound might possess an acidic phosphate group; this was confirmed by the strongly positive reaction for inorganic phosphorus in the hydrochloric acid hydrolysate.

Progressive acid hydrolysis with 1 N hydrochloric acid at 100 °C in sealed tubes yielded useful information on the composition of the paragonial substance. After 30 min of hydrolysis, free ethanolamine was liberated[1] (high-voltage electrophoresis at pH 1.9) in maximum yield, with an acidic phosphorus-containing, ninhydrin-negative residue remaining close to the origin. By analogy with L-α-glycerophosphorylethanolamine (Schmidt et al., 1953) this suggested the presence of esterified phosphorylethanolamine.

The paragonial substance itself was nonreducing toward alkaline ferricyanide, but became maximally reducing after about 90 min of hydrolysis with 1 N hydrochloric acid at 100 °C. Accordingly, a sample was hydrolyzed for 2 h, charged molecules were removed according to Spiro (1966), and the concentrated neutral solution was examined by paper chromatography. Three solvent systems (ethyl acetate–pyridine–water, 12:5:4; 1-butanol–ethanol–water, 10:1:2; 1-butanol–acetic acid–water, 4:1:5) served to identify free galactose and glycerol in the hydrolysate. A second sample treated as above

[1] Free ethanolamine was also liberated following 30 min of hydrolysis with 1 N sodium hydroxide at 100 ° C.

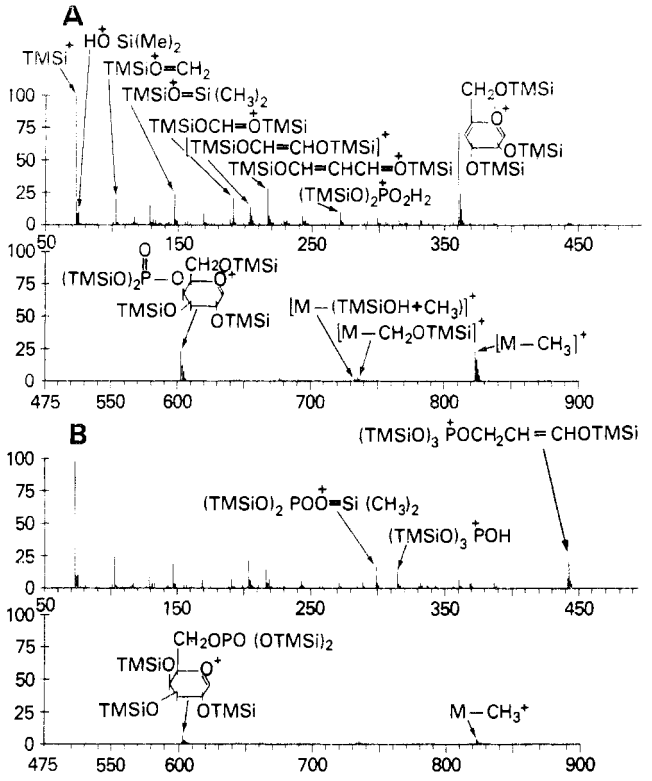


FIGURE 2: Mass spectra of base-hydrolyzed paragonial substance. Ionizing voltage 70 eV. (A) Leading edge of main peak. (B) Trailing edge of main peak.

was reduced and acetylated (Laine et al., 1972), and examined by gas chromatography. Two peaks were obtained, with retention times corresponding to glycerol triacetate and galactitol (dulcitol) hexaacetate, respectively.

Based on the findings thus far, a sample of the paragonial material and separate standards of galactose, glycerol, and ethanolamine were individually treated with 6 N hydrochloric acid at 100 °C for 1 h and these components, together with phosphorus, estimated quantitatively. The molar ratios of phosphorus:galactose:glycerol:ethanolamine in the paragonial substance (corrected for hydrolytic losses from the standards) were found to be 1.0:1.1:1.14:0.96, i.e., in unistoichiometric proportions.

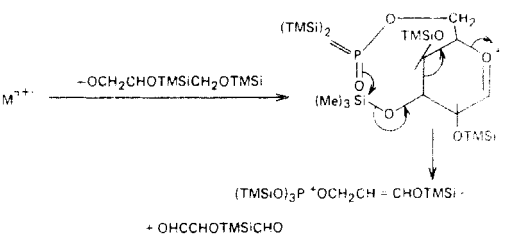
Attempts to cleave the molecule with wheat germ acid phosphatase, or with snake venom phosphodiesterase, were unsuccessful. Similarly, no inorganic phosphate or glycerol was released by incubation with β-galactosidase. On treatment with sodium periodate, 1.9 mol of periodate/mol of phosphorus was taken up, and 1.1 mol of formaldehyde was liberated. The chemical findings thus indicate the paragonial material to be a galactoside which, due to the relatively difficultly hydrolyzable phosphorus, is unlikely to be galactose 1-phosphate, and hence must have either glycerol or ethanolamine attached to C-1. For further characterization, we therefore turned to physical procedures.

Gas Chromatography–Mass Spectrometry. Gas chromatography–mass spectrometry (GC-MS) on 1% OV-17 of BSTFA-treated material after acid hydrolysis (1 N hydrochloric acid, 30 min, 100 °C) confirmed the presence of galactose as its α and β anomers (penta-Me_3Si derivatives) (De Jongh et al., 1969), ethanolamine (bis- and tri-Me_3Si derivatives) (Duncan et al., 1971), phosphate (tri-Me_3Si derivative) (Zinbo and Sherman, 1969), and glycerol (tri-Me_3Si derivative). Traces of α- and β-glyceryl phosphate (tetra-Me_3Si derivatives) (Duncan et al., 1971), later shown to be artifacts, were also observed along with a series of peaks eluting at higher temperatures (210–260 °C). The largest of these eluted at 210 °C (programmed at 10 °C/min), or about where *n*-$C_{23}H_{48}$ would be expected. This compound was a trimethylsilylated hexahydroxy derivative, since its molecular weight (judged from the $M^+ - CH_3$ ions) shifted from 686 to 740 on substitution of BSTFA-d_9 for the former reagent. It showed intense ions at *m/e* 204 and 217 typical of Me_3Si polyols (Zinbo and Sherman, 1971), and subtraction of the contribution to the molecular weight of the trimethylsilyl groups (6 × 72) revealed its basic molecular weight to be 254, suggesting the formula $C_9H_{18}O_8$ corresponding to a glyceryl galactose. For the reasons stated below, the glycerol is likely to be attached to the sugar C-1 position in this degradation product.

On basic hydrolysis (0.1 N sodium hydroxide, 100 °C, 1 h) and treatment with BSTFA, a large GC peak accompanied by early- and late-eluting satellites was observed at 225 °C on 1% OV-17, corresponding to *n*-$C_{27.3}$. Mass spectra of the three peaks showed molecular weights (judged from the $M^+ - 15$ ions) of 838 shifting to 901 on deuteration with BSTFA-d_9. They therefore represent derivatives of heptahydroxy compounds of mol wt 334, suggesting a series of isomeric phosphate esters (254 + 98 − 18 = 334) of the aforementioned galactosylglycerol. The fragmentation patterns of the first peak, the leading edge of the main peak, and the last peak were all very similar (Figure 2A) and about what one might expect for such a compound (Zinbo and Sherman, 1971). In particular, loss of the glyceryloxy substituent to form the stable tetrahydropyrilium ion *m/e* 603 (Figure 2A) also suggests its attachment at C-1. Additional loss of the elements of $(Me_3SiO)_2PO_2H$ probably gives rise to the intense peak at *m/e* 361. If the phosphate residue was instead attached to the glyceryl moiety on the anomeric carbon, formation of the pyrilium ion would be expected to result in an ion at *m/e* 451. A peak is indeed present at this mass but its low intensity indicates that the phosphate group is located elsewhere in this component of the hydrolysate. When the top or trailing edge of the main GC peak is examined, its mass spectrum shows an entirely different fragmentation pattern (Figure 2B). The intense ion at *m/e* 361 is replaced by an important ion at *m/e* 443 and the intensities of the phosphate-containing ions at *m/e* 299, 315, 370, and 387 described by Zinbo and Sherman (1971) are greatly increased in intensity.

We note that in the five available spectra of the 6-phosphate ester of galactopyranose (Markey et al.) a similar ion is observed, and we take this fact to indicate that this component of our mixture is indeed the 6-phosphate ester of the galactosylglycerol moiety. In these spectra the intensity of the *m/e* 443 ion varies from 1 to 39% of the base peak. This may reflect a difference in experimental conditions, but we are inclined to believe that it indicates the presence of other unresolved isomeric phosphates. Migration of phosphate between C-3, C-4, and C-6 may be facile in galactose where the involved groups all have the cis orientation. The ion at *m/e* 443, in fact, contains four Me_3Si groups (BSTFA-d_9) in our compound, and it may be formed from the tetrahydropyrilium ion by the following

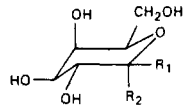
SCHEME I



sort of process (or its one-electron equivalent from M^+). Ample precedent exists for migration of the Me_3Si group to the phosphate moiety under electron ionization conditions (Zinbo and Sherman, 1971).

Regardless of the exact structures of these components of the hydrolysate, it is clear that the alkaline conditions of the hydrolysis have resulted in formation of several isomeric phosphate esters and this information cannot be used reliably to locate the original position of the phosphate ester, although it suggests it is not on the glycerol moiety.

NMR Spectra. ^1H and ^{31}P NMR, besides confirming the above general structural features, established the galactopyranose ring, located the 2-aminoethyl phosphate ester at C-4 of the sugar, and, in addition, proved that the hemiacetal linkage was β to the glycerol moiety. Samples of α-(D-galactopyranosyl)-1-D-glycerol (**1**) and a mixture of 1-*O*-(β-D-galactopyranosyl)-D- and -L-glycerols (**1a**) were important



1 (R_1 = H, R_2 = $OCH_2CHOHCH_2OH$)
1a (R_1 = $OCH_2CHOHCH_2OH$, R_2 = H)

models in deciding this latter point. Both anomers showed a complex signal area at δ 3.70–4.30 (100 MHz in D_2O using Me_4SP as internal reference) attributed to all hydrogens except that on the hemiacetal carbon (C-1). In the case of the α form (**1**) this proton showed a doublet at δ 4.94 (J_{H_1,H_2} = 4 Hz), while the corresponding proton in the β form (**1a**) was shifted upfield to δ 4.68 and showed stronger coupling (J_{H_1,H_2} = 7.5 Hz) (Lemieux and Stevens, 1966). Confirming this, we find the H-1 of α-D-galactopyranoside at δ 5.50 (J_{H_1,H_2} = 3 Hz) and the β form at δ 4.58 (J_{H_1,H_2} = 9.2 Hz). Similarly, α-D-glucopyranosyl phosphate, having the same configuration as galactose at C-1 and C-2, shows δ 5.43 (J = 3.4 Hz) and its β anomer δ 4.89 (J_{H_1,H_2} = 7.5 Hz) for the C-1 proton. The presence of two compounds in **1a** is confirmed by a slight broadening of the anomeric proton resonance relative to that of the pure α anomer.

Based on the above, the C-1 hydrogen of the substance (Figure 3A) is assigned the β configuration, since it appears as a doublet at δ 4.54 (J_{H_1,H_2} = 7.5 Hz). This downfield region is complicated by another ring hydrogen appearing as a doublet of doublets centered at δ 4.57. Phosphorus-31 decoupling experiments (Figure 3B) show that it is coupled to the ^{31}P ($J_{P,H}$ = 9.1 Hz), and its downfield shift from the other ring hydrogens is appropriate for the electron-withdrawing effect of the phosphate ester. The ^{31}P-decoupling experiment also confirms assignments of the α- and β-methylene groups of the ethanolamine side chain at δ 4.26 ($J_{P,H\alpha}$ = 5.5 Hz) and δ 3.36 (J_{H_α,H_β} = 5.1 Hz, $J_{P,H_\beta} \sim$ 0.5 Hz), respectively. Spectra of 1-aminoethanol in D_2O at pD 6 had resonances at δ 3.13 (t) and δ 3.80 (t) (^3J = 5.1 Hz), while *O*-phosphorylethanolamine at the same pD showed resonances at δ 3.28 (t) ($^3J_{H,H}$ = 4.95 Hz, $^4J_{P,H} \sim$ 0.5 Hz) and δ 4.11 ($^3J_{P,H}$ = 5.5 Hz).

The location of the phosphorus-coupled proton on the ring and hence the position of the phosphate group were determined as follows. Irradiation of H-1 at δ 4.54 (Figure 3B) causes a quartet at δ 3.65, previously obscured by other peaks in this region, to appear as one wing of a clearly visible AB pattern (J = 10.0 Hz) assigned to H-2. The other wing (δ 3.82, J = 10.0 Hz), necessarily due to H-3, is revealed when the phosphate-coupled proton at δ 4.57 is irradiated. This proton, and therefore the phosphate group, must be attached to C-4. This

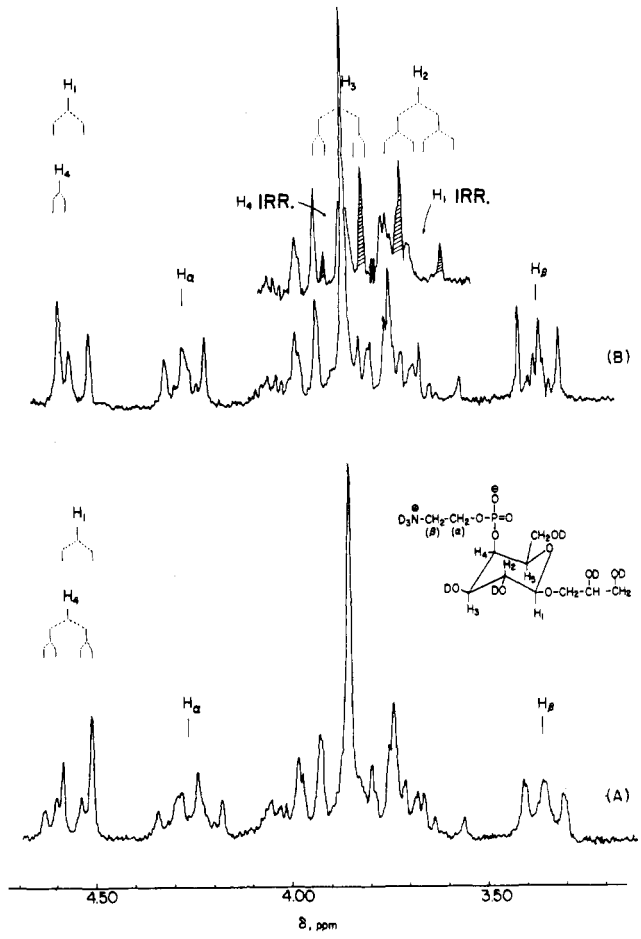
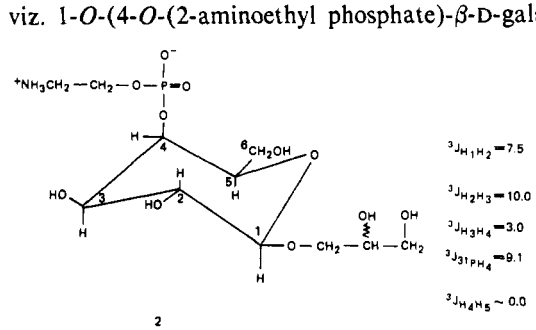


FIGURE 3: ^1H NMR spectra of paragonial substance (~15 mg/0.5 mL of D_2O). (A) Normal spectrum. (B) Lower, ^{31}P-decoupled ^1H spectrum; upper, ^{31}P and either H-1 or H-4 decoupled ^1H spectra.

further establishes the presence of the galactopyranose rather than galactofuranose ring, since the latter requires the ester bridge to be attached to this point. No measurable spin–spin coupling is found between H-4 and H-5. This may be due to the effect of substituent electron negativities of the phosphate and ring oxygen, combined with the cis configuration of these protons (Jackman and Sternhell, 1969).

With the ring identified as being in the galactopyranose form, the aforementioned liberation of 1 mol of formaldehyde after consumption of 2 mol of periodate/mol of phosphorus is explained only if the glycerol is linked to the sugar through its own C-1 carbon; it is thus itself the source of the formaldehyde. If the sugar were in the less familiar furanose form, the formaldehyde might be derived either from the C-6 of the sugar or the glycerol, depending upon the location of the phosphate and how the glycerol is linked.

These facts establish the structure of the paragonial material as **2** viz. 1-*O*-(4-*O*-(2-aminoethyl phosphate)-β-D-galacto-



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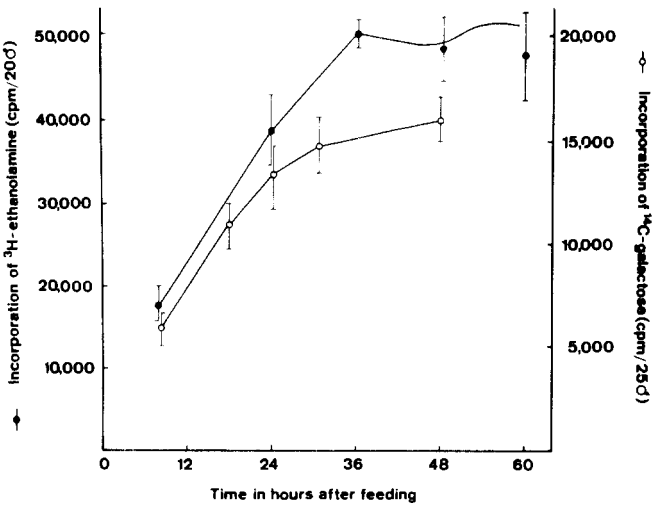

FIGURE 4: Incorporation of dietary [^{14}C]galactose (O-O) and [^{3}H]-ethanolamine (●-●) into paragonial galactoside by 8-day-old male *D. melanogaster* flies. Each point is the mean of three separate determinations.

pyranosyl)-*x*-glycerol. The observed large J_{H_1,H_2} and J_{H_2,H_3} (7.5 and 10.0 Hz, respectively) as well as the assigned β linkage at C-1 suggest that in aqueous solution the galactose moiety exists predominately in the conformation shown. The configuration at the central glycerol carbon remains to be established.

To test the unlikely possibility that the compound was a preparative artifact produced by a Dowex 50 catalyzed interaction between the glycerophosphoryloethanolamine in the initial extract and putative free 1-*O*-(β-D-galactopyranosyl)-glycerol (which if present would have been undetected on the amino acid analyzer), we performed the following experiment. Three milligrams of β-methyl D-glucoside and 3.0 mg of L-α-glycerophosphorylethanolamine were dissolved in 50% (v/v) aqueous methanol, and then treated exactly as described above for purification of the paragonial material. Aside from traces of impurities in the initial compound(s), the only ninhydrin-positive material eluting from the Dowex 50W-X4 column was the original α-glycerophosphorylethanolamine, with no indication of any transfer of the phosphorylethanolamine moiety. Aside from the improbable transphosphorylation of phosphorus to solely the axial C-4 position, this experiment, together with the occurrence of the paragonial galactoside in extracts subjected to only electrophoresis and chromatography, shows that the material is not an artifact.

In Vivo Labeling of the Paragonial Material. As illustrated in Figure 4, radioactive galactose and ethanolamine were both incorporated into the paragonial material when ingested by adult male flies. The isotopes were rapidly incorporated during the initial 24 h after feeding and maximal labeling was attained after about 36 h. Since the radioactivity did not decline during the ensuing 12-24 h, it would appear that in vivo the molecule is metabolically quite stable. These findings are clearly consistent with the proposed structure.

Discussion

The chemical structure of the *D. melanogaster* paragonial material appears to be unique in two respects. Thus, although 1-4 linked galactose polysaccharides in the form of galactans are well known (Aspinall, 1970), this appears to be the first report of a naturally occurring, free, 1,4-disubstituted galactoside. Secondly, while various galactosylglycerols from plant sources have been described (e.g. Colin, 1937; Putnam and Hassid, 1954; Carter et al., 1956; Su and Hassid, 1962), we have not found any previous reference to galactose esterified to phosphorylethanolamine in nature. It is of comparative interest that an unusual 1-*O*-alkyl-2-*O*-acyl-3-(3-thio-β-galactosyl)glycerol has been isolated from seminal fluid of the boar (Yamakawa, 1973).

The *D. melanogaster* galactoside is synthesized by, and accumulates in, exclusively the paragonial glands (Chen and Diem, 1961; Chen and Bühler, 1970). In vitro synthesis, involving the incorporation of labeled ethanolamine, has also been demonstrated (von Wyl, 1974). The compound is transferred from male to female flies during copulation, and can be detected in mated females but not in virgins. In unpublished experiments, when *D. melanogaster* males labeled with [^{3}H]ethanolamine were mated with virgin females, up to 60% of the radioactivity was transferred to the female in a single mating. Not all species of *Drosophila* synthesize the galactoside; in a survey of eight species, an acidic peak on the amino acid analyzer identical in elution time to that from *D. melanogaster* was observed only in *D. simulans, D. ananassae,* and *D. busckii* (Chen and Baumann, 1972).

The biological function(s) of the galactoside is still equivocal. In earlier experiments, injection of the paragonial material isolated directly from the amino acid analyzer into virgin females stimulated oviposition (Chen and Bühler, 1970). However, according to Burckhardt (1975), injection of material purified as described above did not yield consistent stimulatory effects. Further experiments on injection of the purified galactoside under carefully controlled conditions are now under way. Baumann (1974a,b) reported the presence in *D. funebris* paragonial glands of a peptide, PS-1, which reduces female receptivity, and a glycine derivative, PS-2, which stimulates egg laying. Neither of these compounds has been detected in *D. melanogaster,* while the galactoside is absent from *D. funebris.* It thus appears that paragonial compounds effecting behavioral and physiological changes in mated female flies may vary among different *Drosophila* species, and thus serve as a selective mechanism for intraspecific mating.

Acknowledgment

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