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Gadusol, an enolic derivative of cyclohexane-1,3-dione present in the roes of cod and other marine fish

Isolation, properties and occurrence compared with ascorbic acid

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Gadusol, $C_8H_{12}O_6$, has been isolated from roes of the cod (*Gadus morhua* L.), i.e., ovaries that contain ripe eggs just before spawning. The concentration is about 4 g/kg dry wt. It has been identified as 1,4,5-trihydroxy-5-hydroxymethyl-2-methoxycyclohex-1-en-3-one and this structure was confirmed by synthesis of the anhydro tetra-acetate derivative from methyl 3,5-diacetoxy-4-methoxybenzoate. Concentrations of gadusol in the roes of other marine teleost fish examined are of the same order as in cod roes. Gadusol has some properties similar to ascorbic acid and both compounds, after oxidation, react with 2,4-dinitrophenylhydrazine in the commonly-used assay procedure for ascorbic acid. Specific assays showed that the concentrations of gadusol in the roes of marine fish are severalfold greater than those of ascorbic acid. Gadusol is structurally related to the mycosporines previously reported from a number of different organisms.

Mature female cod produce each year several million eggs. Each egg contains a supply of nutrient for the developing embryo and, after hatching, the larva continues to depend on its attached yolk sac for its food during early development. Before spawning, the roes represent 15–20% of the wet weight of the female cod. Most of the dry matter of the roe is a specific lipoprotein (lipovitellin), which has been shown to be synthesized in the liver, transported in the serum and deposited in the roe (Plack & Fraser, 1971; Plack *et al.*, 1971). In the course of that work, a saline extract of cod roes was subjected to gel filtration on a column of Sephadex G-200. The absorbance at 280nm of the fractions when plotted against fraction number gave two separate peaks of about equal area. The first was due to material of high molecular weight, mostly lipovitellin, and the second to compounds of low molecular weight with u.v. absorbance.

The main low-molecular-weight u.v.-absorbing compound has now been isolated and named gadusol. Evidence is presented here of its chemical structure and its occurrence, compared with ascorbic acid, in the roes of some marine teleost fish. A preliminary report of the structure of gadusol has been published previously (Grant *et al.*, 1980).

Experimental

Animals

Roes, i.e. ovaries containing ripe eggs, were taken from the following animals: cod (*Gadus morhua* L.), haddock [*Melanogrammus aeglefinus* (L.)], common dab [*Limanda limanda* (L.)], long rough dab [*Hippoglossoides platessa* (Fabricius)], flounder [*Platichthys flesus* (L.)], plaice (*Pleuronectes platessa* L.) and lemon sole [*Microstomus kitt* (Walbaum)].

Maturation coefficient (M.C., or gonado-somatic index) is defined as (wt. of gonads × 100)/(wt. of whole fish). In following the deposition of gadusol and of ascorbic acid in the developing ovaries of cod, fish weighing between 1.74 and 4.66kg were used. The amounts found per pair of ovaries and the dry wt. of the ovaries were multiplied by 3.00/(wt. of fish in kg) to give calculated values for the ovaries of a standard fish of 3kg wt. with the same maturation coefficient.

Chemicals

Analytical-Reagent-grade chemicals were used whenever available. Metaphosphoric acid sticks (BDH, Poole, Dorset, U.K.) contain approximately

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60% HPO_3; they were washed before use until transparent and made up as a 3% (w/v) solution to give approx. 1.8% (w/v) metaphosphoric acid. The cyclohexane-1,3-dione was from Aldrich Chemical Co. (Gillingham, Dorset, U.K.).

Purification of gadusol

Roes from mature female cod were stored in deep freeze at −20°C. After thawing, the egg mass was scraped from the inside of the ovary membrane and batches of about 1kg were used for each preparation. Portions of 100g were homogenized with a top-drive homogenizer (MSE, Crawley, Sussex, U.K.) for 2min to rupture the eggs, and the viscous mixture was added to 400ml of ethanol and homogenized further with an Ultra-Turrax homogenizer (The Scientific Instrument Centre, London, U.K.) to extract the tissue. After being left overnight at 6°C, the mixture was filtered and the solid residue was washed with 1 litre of 80% (v/v) ethanol/water, re-homogenized with 2 litres of 80% (v/v) ethanol/water and filtered again. The volume of the combined filtrates was then reduced *in vacuo* at 40°C with a rotary film evaporator to about 100ml of a red liquid. This residue was transferred to a 2-litre separating funnel together with successive flask washings of 100ml of ethanol, two lots of 100ml of chloroform and two lots of 100ml of water. After shaking, the mixture was allowed to settle overnight to a dark-red chloroform layer and a pale-yellow ethanol/water layer. The latter was washed with 200ml of chloroform and reduced in volume to about 30ml. At this stage the extract contained about 12g of solids, of which about 0.5g was gadusol and the remainder largely amino acids. Ion-exchange chromatography on Dowex 50 (X8; 200–400 mesh; H^+ form; column dimensions 18cm × 4.5cm diam.) and elution with water allowed the gadusol to pass through unretarded. Most of the impurities were retained on the resin. The fractions containing gadusol, recognized by their u.v.-absorption curves, were combined and repeatedly evaporated to dryness from water and then from ethanol to remove most of the HCl. The residue was dissolved in 5ml of 0.5M-acetic acid.

This solution of gadusol was applied to a column (28cm × 1.8cm diam.) of Dowex 1 (X8; 200–400 mesh; acetate form) (Hirs *et al.*, 1954), and eluted with 0.5M-acetic acid at about 12ml/h. The gadusol was identified in the 7ml fractions by its absorption maximum of 296nm at pH7 or above, and the curve of absorption against fraction number frequently showed two peaks, the main one at an elution volume of about 90ml and a smaller one at an elution volume varying from 140 to 240ml. All these fractions were combined, taken to dryness, dissolved in 0.5M-acetic acid and applied to a second similar column of Dowex 1 resin. The plot of A_{296} against fraction number now had a single symmetrical peak with its maximum corresponding to an elution volume of about 280ml. (In contrast, pure ascorbic acid on a similar Dowex 1 column was eluted with the maximum concentration at an elution volume of about 1730ml.)

Removal of water and acetic acid from the combined gadusol fractions gave a colourless viscous oil to which ethanol was added to give a concentration of 100mg of oil/ml of ethanol. The mixture was stored overnight under N_2 at 6°C to give a white amorphous solid, which was dissolved in hot ethanol, filtered hot through a no. 3 sintered funnel and kept at 6°C overnight to yield a white solid. The last procedure was repeated and the final solid was washed with cold ethanol and cold diethyl ether, dried for 72h in a vacuum desiccator over P_2O_5 and sealed into ampoules under vacuum.

Reaction of gadusol with acetic anhydride

Gadusol (40mg) was refluxed with 20mg of anhydrous sodium acetate in 1ml of acetic anhydride for 2.3h. The mixture was cooled, added to 5ml of water, stirred for 1.5h and extracted with chloroform and diethyl ether. The combined extracts were shaken successively with 2M-$NaHCO_3$, water and brine, dried over anhydrous $MgSO_4$ and evaporated to leave a pale-brown oil, which partly crystallized *in vacuo*. After trituration with aq. methanol (1:1, v/v) the product crystallized from light petroleum (b.p. 60–80°C).

Preparation of 2,3,5-triacetoxy-1-acetoxymethyl-4-methoxybenzene (Fig. 1, II)

To a stirred suspension of lithium aluminium hydride (2.1g) in 80ml of dry diethyl ether was added, during 1h, a solution of 7.5g of methyl 3,5-diacetoxy-4-methoxybenzoate (Fig. 1, III; prepared by the method of Fischer *et al.*, 1918) in 90ml of diethyl ether. The mixture was refluxed for 2.5h. After cooling, 1ml of ethyl acetate in 10ml of diethyl ether was added and refluxing was continued for 10min. After cooling again, 2M-H_2SO_4 was added and the layers were separated. The aqueous phase was saturated with NaCl and extracted several times with diethyl ether and then with ethyl acetate. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to leave a residue of 3,5-dihydroxy-4-methoxybenzyl alcohol (Fig. 1, IV), which crystallized from toluene/ethyl acetate. The product had m.p. 164°C (Found: C, 56.2; H, 6.2%; M^+, 170.0581; $C_8H_9O_4$ requires C, 56.5; H, 5.9%; *M*, 170.0579); δ (p.p.m.) [(^{2}H)methanol] 6.38 (2H, s, Ar*H*), 4.41 (2H, s, CH_2), 3.77 (3H, s, OCH_3); *m/e* (%) 170 (100), 153 (18), 141 (7), 127 (32), 109 (56), 81 (32).

The alcohol (0.4g), dissolved in 10ml of methanol and 40ml of 0.17M-KH_2PO_4, was added, all at once,

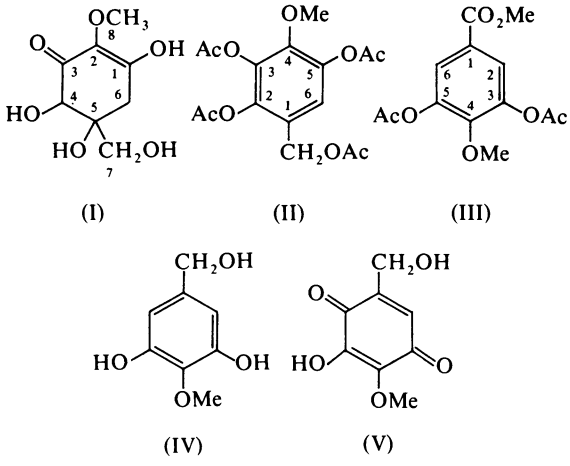

Fig. 1. *Structures of gadusol and related synthetic compounds*

I, gadusol (1,4,5-trihydroxy-5-hydroxymethyl-2-methoxycyclohex-1-en-3-one); II, tetra-acetate of anhydrogadusol (2,3,5-triacetoxy-1-acetoxymethyl-4-methoxybenzene); III, methyl 3,5-diacetoxy-4-methoxybenzoate; IV, 3,5-dihydroxy-4-methoxybenzyl alcohol; V, 2-hydroxy-6-hydroxymethyl-3-methoxy-1,4-benzoquinone.

to a solution of 1.4g of Fremy's salt (potassium nitrosulphonate) in 60ml of water. After 1h the solution was extracted with ethyl acetate. NaCl (25g) was then added and the solution repeatedly extracted with ethyl acetate. The combined orange extracts were shaken with 1M-$NaHCO_3$, which became purple. The aqueous phase was removed, acidified, saturated with NaCl and extracted with ethyl acetate. After drying with anhydrous $MgSO_4$, the solvent was removed by evaporation. The residual purple–brown quinone (105mg; presumably structure V in Fig. 1) was refluxed for 20min in 1ml of acetic anhydride containing 100mg of zinc dust and 1 drop of pyridine, and decanted on to ice. The zinc residue was extracted with two lots of 1ml of hot acetic acid and the extract added to the ice. The crude product was extracted with chloroform and diethyl ether, washed with 1M-$NaHCO_3$ and water, and dried over anhydrous $MgSO_4$. After evaporation, a thick yellow oil remained and was purified by preparative t.l.c. (twice) on silica gel with chloroform as the eluent, triturated with aq. methanol (1:1, v/v) and finally crystallized from light petroleum.

Determination of ascorbic acid

The photometric 2,6-dichlorophenol-indophenol method described by Roe (1954) was used with minor modifications. Samples of ovaries were extracted by removing 2g of tissue, primarily eggs, from the inside and homogenizing with 8ml of 1.8% (w/v) HPO_3 in a Potter–Elvehjem homogenizer. After 30min of extraction, the homogenate was centrifuged at 100000*g* for 30min and the clear supernatant was used. With liver and muscle, samples of 10g were homogenized with 40ml of HPO_3 solution in an MSE top-drive homogenizer and, after being left for 30min for extraction, the homogenate was filtered through a Whatman no. 5 filter paper.

Measurements were carried out in cuvettes of 4cm light path with a Pye–Unicam SP. 800 recording spectrophotometer set for a constant wavelength of 515nm and recording absorbance against time. On mixing the 2,6-dichlorophenol-indophenol solution and the ascorbic acid sample, reduction of the dye, as shown by the fall in absorbance, occurred in seconds. Standards containing from 5 to 50μg of ascorbic acid gave a straight line for the plot of fall in absorbance against weight corresponding to 0.0192/μg of ascorbic acid in the cuvette, and this value was used to calculate concentrations.

Gadusol also reduces 2,6-dichlorophenol-indophenol, but at a very much lower rate, taking hours rather than seconds. Provided the absorbance in the ascorbic acid assay is read as rapidly as possible, there is no significant interference by gadusol.

Determination of gadusol

The extracts prepared with metaphosphoric acid for the determination of ascorbic acid were used, and gave results similar to those obtained with aqueous extracts treated with trichloroacetic acid to remove protein. Each extract was diluted with water to give a suitable maximum absorbance. A 3ml sample of the dilution in a cuvette of 1cm light path was made basic (pH approx. 8) by the addition of a few drops of 2M-NaOH and the absorption curve between 225 and 350nm determined wihh a Pye–Unicam SP. 800 recording spectrophotometer. The same sample was then made acidic (pH approx. 1) by the addition of one drop of 10M-HCl and the new absorption curve recorded. Concentrations of gadusol were calculated from the relationship A_{296} (basic − acidic) = 0.0914 for a concentration of 1μg/ml.

With samples containing 1mg of gadusol/g wet wt. of tissue the absorption curves obtained were similar to those of the pure compound. At lower concentrations, other absorbing material in the extracts obscured the curves, but a difference curve, obtained by subtracting the acidic absorption curve from the corresponding basic curve, with a peak at about 300nm, was regarded as proof of the presence of gadusol, and the lower limit of detection was about 10μg/g wet wt. of tissue. Much of the

extraneous u.v. absorption could be removed by chromatography of the metaphosphoric acid extract on Dowex 50 (X8; 200–400 mesh; H^+ form; column dimensions 5 cm × 1 cm diam.) and elution with water. Gadusol was not retarded.

Ascorbic acid interferes little with this assay and a value for A_{296} (basic − acidic) = 0.0064 was found for a solution of the pure compound at 1 μg/ml.

Other methods

Titration curves of an aqueous solution of gadusol (1 ml containing about 15 mg) against 0.2 M-NaOH were obtained with a Radiometer pH meter PHM 25, Autotitrator TTT 11 and Titrigraph SBR2.

A Perkin–Elmer 555 recording spectrophotometer was used to give absorption curves of aqueous solutions of pure gadusol. Values of absorbance were determined manually with the same instrument and molar absorption coefficients were based on weighed quantities of gadusol.

The behaviour of gadusol in the 2,4-dinitrophenylhydrazine assay of ascorbic acid was examined by the method of Roe & Kuether (1943) as modified by Ikeda *et al.* (1963*a*).

For the bioassay of antiscorbutic activity with guinea-pigs, three groups, each of five animals, were used. All the animals were given a diet free of ascorbic acid. The first group received no supplement, the second group 1 mg of ascorbic acid each day by mouth and the third group received 1 mg of gadusol each day by mouth.

Results

Structure and properties of gadusol

Gadusol was isolated from extracts of cod roes by chromatography on Dowex 50 (H^+ form) followed by chromatography on Dowex 1 (acetate form). Subsequently gadusol was obtained as a white amorphous powder on cooling a concentrated solution in ethanol. It melted at 178–179°C and sublimed unchanged at about 120°C and at 4 Pa. Gadusol was soluble in water and slightly soluble in ethanol, ethyl acetate and pyridine. It gave a single spot after t.l.c. on silica-gel plates with ethanol/water (9:1, v/v; R_F 0.76) and butan-1-ol/acetic acid/water (12:3:5, by vol.; R_F 0.33). Spots were detected with either ethanolic $FeCl_3$ (instant wine-red colour) or 2,4-dinitrophenylhydrazine in 2 M-HCl (yellow–brown colour after 12 h).

Mass spectrometry gave M^+ 204.0633 ($C_8H_{12}O_6$ requires 204.0633) with prominent lines at *m/e* (%), 204 (67), 186 (3), 173 (71), 168 (7), 156 (100) and 141 (50). By titration with alkali and assumption of one titratable group per molecule (pK_a 4.25), the purity of three separate preparations was greater than 99%.

Aqueous solutions were optically active {$[\alpha]_D^{23}$ + 100 (*c* 0.27 in water)} and had a distinctive u.v. absorption: $\lambda_{max.}$ 269 nm (ε 12400 litre·mol^{-1}·cm^{-1}) at pH < 2 and $\lambda_{max.}$ 296 nm (ε 21800 litre·mol^{-1}·cm^{-1}) at pH > 7.

The i.r. spectrum (KBr) had $\nu_{max.}$ 3700–2400, 1664 and 1611 cm^{-1}. N.m.r. chemical-shift data were as follows: ^1H δ (p.p.m.) [(^2H)methanol] 4.10 (1H, s, C*H*OH), 3.69 and 3.48 (each 1H, dd, *J* 12 Hz, CH_2OH), 3.60 (3H, s, OCH_3), 2.78 and 2.38 (each 1H, dd, *J* 18 Hz, ring CH_2); ^{13}C δ (p.p.m.)* [(^2H)methanol] 179.31 (s, C-1 and C-3), 134.68 (s, C-2), 75.15 (s, C-5), 74.83 (d, C-4), 65.48 (t, C-7), 60.23 (q, C-8), 39.18 (t, C-6).

These data are consistent with the enolized β-diketone structure 1,4,5-trihydroxy-5-hydroxymethyl-2-methoxycyclohex-1-en-3-one (Fig. 1, I), for which we use the trivial name gadusol.

Reaction of gadusol with acetic anhydride gave a benzenoid derivative, m.p. 92–93°C (Found: M^+ 354.0954, $C_{16}H_{18}O_9$ requires 354.0950; *m/e* (%) 354 (0.8), 312 (5), 270 (7), 211 (6), 210 (56), 168 (100); $\lambda_{max.}$ (chloroform) 247 (shoulder), 267 (ε 390 and 780 litre·mol^{-1}·cm^{-1}); $\nu_{max.}$ (KBr) 1738, 1768 cm^{-1}; δ (p.p.m.) [(^2H)chloroform] 7.08 (1H, s, Ar*H*), 5.00 (2H, s, CH_2), 3.82 (3H, s, OCH_3), 2.30 (3H, s, OCOCH_3), 2.27 (6H, s, 2 × OCOCH_3), 2.03 (3H, s, OCOCH_3). These data were identical in all respects with those obtained with 2,3,5-triacetoxy-1-acetoxymethyl-4-methoxybenzene (Fig. 1, II), that had been prepared by synthetic procedures from methyl 3,5-diacetoxy-4-methoxybenzoate (Fig. 1, III). On this basis, acetylation of gadusol is accompanied by dehydration and aromatization of the original ring system to yield compound II (Fig. 1).

Gadusol reduced solutions of cupric salts to cuprous, potassium ferricyanide to ferrocyanide and reduced 2,6-dichlorophenol-indophenol very slowly. Aqueous I_2 in KI, and bromine water, were decolorized rapidly, with loss of the u.v. absorption of gadusol. These reactions could not be reversed by reducing agents such as sodium borohydride. A biological assay with guinea-pigs showed that gadusol had no antiscorbutic activity

Occurrence of gadusol compared with ascorbic acid

In the dinitrophenylhydrazine test for ascorbic acid, gadusol gave a value 42% of that given by the same weight of ascorbic acid. The gadusol derivative had two absorption maxima at 450 and 471 nm instead of the maximum for ascorbic acid, 530 nm, that is used in the assay. The 2,6-dichlorophenol-indophenol assay was therefore used in the present work to avoid interference by gadusol.

* Some signals were incorrectly assigned in the preliminary report (Grant *et al.*, 1980).

Table 1. *Mean concentrations* (±S.E.M.) *of gadusol and of ascorbic acid in roes of marine fish*

Fish	Number of samples	Gadusol (mg/g dry wt.)	Ascorbic acid (mg/g dry wt.)
Cod	6	4.30 ± 0.295	0.51 ± 0.037
Haddock	9	3.69 ± 0.254	0.70 ± 0.045
Common dab	4	4.34 ± 0.304	1.05 ± 0.263
Long rough dab	3	2.90 ± 0.232	0.38 ± 0.029
Flounder	3	4.19 ± 0.639	0.75 ± 0.090
Plaice	2	3.47	0.44
Lemon sole	1	1.21	2.38

Table 2. *Mean concentrations* (± S.E.M.) *of gadusol and of ascorbic acid in cod tissues*

Fish	Tissue	Number of samples	Gadusol (mg/g dry wt.)	Ascorbic acid (mg/g dry wt.)
Immature female (M.C. < 0.35)	Ovaries	6	0.10 ± 0.044	1.36 ± 0.108
	Liver	5	0.01 ± 0.008	0.05 ± 0.008
Mature female (M.C. > 10.0)	Ovaries	6	4.30 ± 0.295	0.51 ± 0.037
	Liver	6	0.03 ± 0.016	0.04 ± 0.004
	Muscle	1	0.01	0.04
	Whole blood	1	0.02 (mg/ml)	0
Mature male (M.C. > 7.3)	Testes	3	0	0.24 ± 0.017
	Liver	3	0.02 ± 0.015	0.04 ± 0.003

Table 1 shows that gadusol was found in the roes of a number of marine teleost fish and, with the exception of lemon sole, its concentration was severalfold greater than that of ascorbic acid.

Examination of other tissues of the cod (Table 2) showed that only ovaries with ripe eggs contained any quantity of the compound. None was found in testes and the quantities in liver and muscle were at the limit of detectability. However, a small concentration was present in blood.

Three cod roes from fish caught off the west coast of Scotland gave mean values of 3.04 mg of gadusol and 0.22 mg of ascorbic acid/g dry wt. A haddock roe from the same region had 3.57 and 0.60 mg/g dry wt. respectively. Ethanol extracts from the roe of a cod of the Arcto-Norwegian stock had 0.38 mg of gadusol/g wet wt. (about 1.4 mg/g dry wt.) and similar extracts from two cod from Balsfjorden contained 0.55 and 0.56 mg of gadusol/g wet wt. (about 2.0 and 2.1 mg/g dry wt.).

Fig. 2 shows the total amounts of gadusol and of ascorbic acid in the ovaries of standard cod of 3 kg wet wt. during the course of egg development in the ovaries, represented here by the increase in weight of

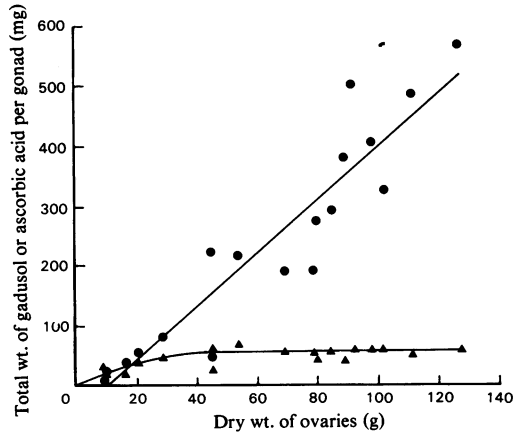

Fig. 2. *Amounts of gadusol* (●) *and of ascorbic acid* (▲) *in the ovaries of cod plotted against dry wt. of ovaries* Values for individual fish were recalculated for a standard cod of wet wt. 3 kg with the same maturation coefficient (see the Experimental section). The straight line shown for gadusol, $y = 4.42x - 43.44$, was obtained by the method of least squares.

the ovaries. The quantity of ascorbic acid increased during the early stages and then remained constant at about 50mg, whereas the quantity of gadusol increased approximately linearly during the course of development and the total amount deposited in the ovaries was about 500mg for a fish of 3kg wet wt.

Discussion

The chemical structure of gadusol, determined primarily by mass spectrometry and n.m.r. and confirmed by synthesis of the anhydro tetra-acetate, is that of an enolized cylcohexane-1,3-dione derivative (Fig. 3, I). In solution this tautomeric compound exists essentially in the enol form, as shown by the u.v. and n.m.r. spectra. With increase in pH the enolate ion is formed, resulting in increases in the wavelength of maximum absorption and in the molar absorption coefficient. Similar spectral changes are found with the isomeric compound, 'spinulosin quinol-hydrate' (Fig. 3, III), a secondary product of fungal metabolism (Yamamoto *et al.*, 1970), as well as with ascorbic acid and cyclohexane-1,3-dione. Gadusol also contains two asymmetric carbon atoms and is optically active. The configurations about the asymmetric carbon atoms are unknown, but the occurrence of a number of isomeric forms may be one explanation for our inability to obtain the compound in a crystalline form from ethanol.

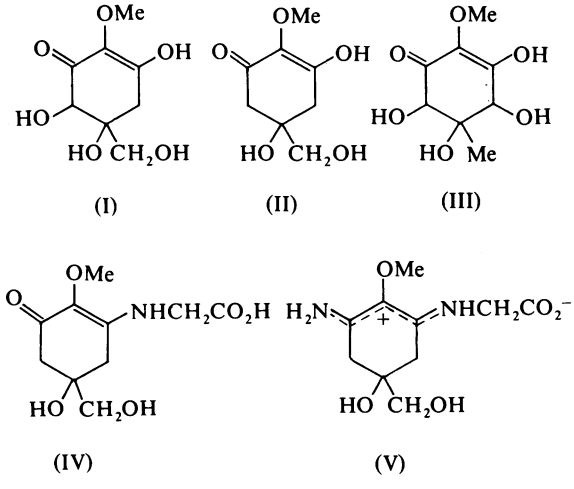

Fig. 3. *Structures of gadusol and of related compounds found to occur naturally*

I, gadusol (1,4,5-trihydroxy-5-hydroxymethyl-2-methoxycyclohex-1-en-3-one); II, 1,5-dihydroxy-5-hydroxymethyl-2-methoxycyclohex-1-en-3-one; III, 'spinulosin quinol-hydrate'; IV, a mycosporine; V, an aminocyclohexenimine.

Previous determinations of ascorbic acid in fish ovaries by the dinitrophenylhydrazine method (Hastings & Spencer, 1952; Ikeda *et al.*, 1963*b*) are likely to be over-estimates due to the presence of gadusol, which also reacts with dinitrophenylhydrazine. We have therefore used methods for the determination of gadusol and ascorbic acid that minimize mutual interference and find that in the roes of most of the marine teleost fish examined, the concentration of gadusol is several times greater than that of ascorbic acid. The difference between the two compounds is further emphasized when the total amounts of gadusol and of ascorbic acid in cod ovaries are followed during gonadal maturation (Fig. 2).

The reason for the accumulation of gadusol in cod roes and its biosynthetic origin remain to be established. If it is derived directly or indirectly from the diet then either gadusol or a precursor must be a relatively common constituent of many species in the marine food chains, since roes from cod caught in different sea areas all contained similar concentrations of gadusol, as did the roes of other marine fish consuming different food organisms. Chioccara *et al.* (1980) have identified gadusol in the eggs of some Mediterranean fish, as well as traces of a similar compound, 1,5-dihydroxy-5-hydroxymethyl-2-methoxycyclohex-1-en-3-one (Fig. 3, II). Possible precursors of gadusol are the structurally related mycosporines (e.g., structure IV in Fig. 3) and aminocyclohexenimines (e.g., structure V in Fig. 3) that have recently been identified in a variety of organisms. Mycosporines have been found in various fungi (Favre-Bonvin *et al.*, 1976, 1980; Arpin *et al.*, 1977; Lunel *et al.*, 1980) and in a zooanthid (Ito & Hirata, 1977; Hirata *et al.*, 1979). Aminocyclohexenimines are found in red algae (Tsujino *et al.*, 1978; Takano *et al.*, 1979), in a zooanthid (Takano *et al.*, 1978*a*,*b*; Hirata *et al.*, 1979) and in mussels (Chioccara *et al.*, 1979), as well as in fish eggs (Chioccara *et al.*, 1980).

The high concentration of gadusol in cod roes (about 4g/kg dry wt.) suggests that the compound has some functional role in embryonic development. Although gadusol has some properties similar to those of ascorbic acid, the inability to reduce oxidized gadusol chemically and the absence of antiscorbutic activity for guinea-pigs indicate that gadusol does not act as a biological substitute for vitamin C.

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