chlorine substitution) in the sequence R = CH_3CH_2-, CH_2ClCH_2-, CCl_3CH_2- owing to a large increase in the activation energy. The activation energies in the ethanolysis of the first two compounds in 53.3 wt.% ethanol-dioxan are, respectively, 11.13 and 13.55 kcal per mole [1,4]. The logarithms of the frequency factor for the three compounds have values varying from 7.04 to 7.66 and hence the entropies of activation differ only insignificantly. The structural effects are thus consistent with the unimolecular carbonium ion mechanism of ethanolysis.

On the basis of the data presented it is possible to estimate the effect of the β,β,β-trichloroethyl group on the hydrolysis mechanism of the corresponding alkoxymethyl esters in dilute acid solutions. The polar substituent constant $\bar{\sigma}$ *, as defined in Ref.[4], for this group is + 2.87. From this value it can be estimated that the rate coefficient for the hydrolysis of β,β,β-trichloroethoxymethyl acetate (VII) by the unimolecular $A_{AL}1$ mechanism in dilute aqueous acid at 25°C should be about 7×10^{-7} l mole^{-1}s^{-1}. As this latter value is of a much lower order of magnitude than the rate coefficients relating to the normal type of acid-catalysed hydrolysis reactions of alkyl acetates (about 10^{-4} l mole^{-1}s^{-1}), *i.e.* reactions taking place by the bimolecular $A_{AC}2$ mechanism, it can be assumed that also the ester (VII) hydrolyses almost exclusively by the latter mechanism. In the case of the corresponding formate, (VI), the conditions for the unimolecular hydrolysis mechanism are even less favourable.

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The Effect of Acetone Treatment on Vitamin A_1-Aldehyde Extracts from Herring Roe

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Plack *et al.*[1] have demonstrated the presence of vitamin A_1 aldehyde in herring ova *(Clupea harengus)* and some other marine teleosts. They investigated several extraction methods, but found that extraction of the eggs with light petroleum followed by addition of ethanol to the blended mixture gave the best results. Pollard and Bieri [2] confirmed some of these findings, but reported that all their samples of herring roe contained only vitamin A_2 aldehyde. They extracted the ova several times with ethanol and isolated the vitamins by hexane extraction of the alcohol extracts.

During studies of the occurrence of vitamin A aldehydes in fish in our laboratory, we applied both the proposed extraction methods on the herring roe *(Clupea harengus)*, and found only vitamin A_1 aldehyde to be present. We were further only able to demonstrate the presence of vitamin A alcohol in these extracts but not the ester. Plack *et al.*[1] estimated the vitamin concentrations by the Carr-Price test, and reported for herring ova 2.4—5.7 μg vitamin A_1 aldehyde per g and 0.3—0.9 μg vitamin A_1 ester and alcohol per g. We have carried out the estimations by spectrophotometric measurements in the ultraviolet and found only 1 μg vitamin A_1 aldehyde and 0.3 μg vitamin A_1 alcohol per g herring roe. The absorption curves of the fractions were plotted, thus establishing the identity of the compounds.

Plack *et al.*[1] studied the effect of acetone treatment of the light-petroleum extracts. They found in liver storage tests in rats that only the acetone-soluble fraction contained biological activity, while the acetone-insoluble fraction showed no activity. In repeated experiments they could only recover about 40 % of the activity originally present in the extracts. This suggested a further investigation of the soluble portion, and the present communication reports some of the findings.

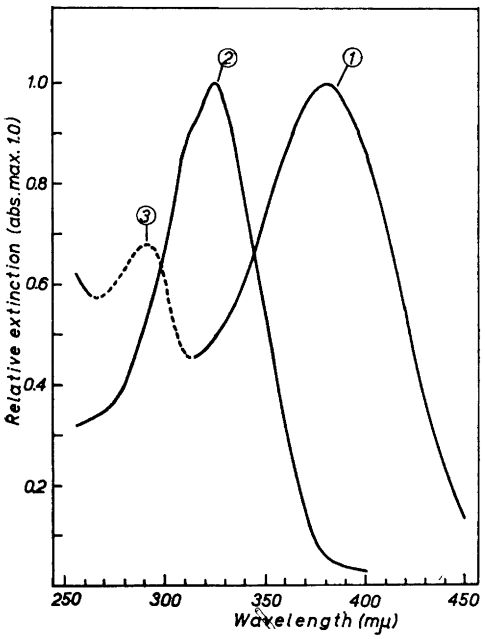

Fig. 1. Absorption curves of vitamin A_1-aldehyde extracted from herring ova (1), and its reduction product vitamin A_1 (2). The dotted band (3) is caused by the presence of α-tocopherol.

Extracts obtained by both the reported methods [1,2] were evaporated to dryness under reduced pressure. The residues were extracted with acetone as described by Plack *et al.*[1] or with the following modification: The residue was dissolved by heating with acetone, and then cooled by partial evaporation in vacuum, whereby a precipitate formed which was insoluble at room temperature. This precipitate was washed several times with cold acetone. The acetone extracts were combined and evaporated to dryness, dissolved in hexane and chromatographed on a soft Al_2O_3-column. The elution was carried out with 2 % (v/v) ethyl ether in hexane. As the aldehyde band approached the end of the column, 10 ml fractions were collected. These fractions were evaporated to dryness, dissolved in ethanol and measured in a Beckman DU spectrophotometer. The "aldehyde" fractions showed absorption curves with maxima between 390—400 mμ. The later fractions had absorption maxima at the highest wavelengths. By the normal procedure (without acetone treatment of the residue), a curve with absorption maximum at 380 mμ, corresponding to vitamin A_1-aldehyde, was observed. Unfortunately vitamin A_1-aldehyde and α-tocopherol present in the extracts move together on this type of column. Thus a band corresponding to α-tocopherol may be observed on the "aldehyde" absorption curves (Fig. 1).

When the different alcohol-solutions were reduced with sodium borohydride [1], the absorption maxima were shifted towards lower wavelengths. The reduction products were readily freed from α-tocopherol by chromatography on soft Al_2O_3. It may be mentioned that the reduction procedure employed did not influence the absorption maximum of α-tocopherol. Vitamin A_1 aldehyde gave a compound with absorption maximum at 325 mμ (vitamin A_1). For all the acetone treated extracts reduction resulted in compounds with absorption maxima at 340—360 mμ. Again the later frac-

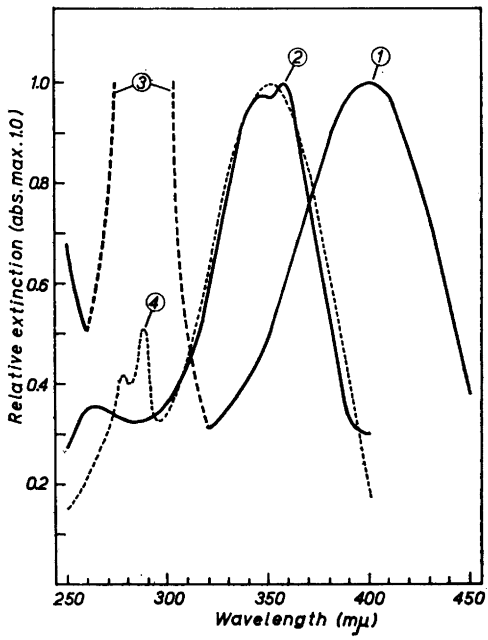

Fig. 2. Absorption curves of compound(s) isolated from acetone treated extracts from herring ova (1), and its reduction product(s) (2). The dotted band (3) is caused by the presence of α-tocopherol. For comparison is plotted vitamin A_2 after Lambertsen & Brækkan[10] (4).

tions showed absorption maxima at the higher wavelengths. A comparison of the fractions indicated the presence of a mixture of two compounds with similar chromatographic properties on the column used. The spectrophotometric data are summarized in Figs. 1 and 2.

The spectrophotometric properties of the fractions obtained were very similar to those reported for oxidation-products obtained by the Oppenauer reaction in the presence of acetone or diethyl ketone [3-5]. Apparently the compounds observed by us are axerophthylidene-acetone [3] and the so-called "C_{20}-aldehyde" [4,5], and their reduction products. In Table 1 are summarized data reported in the literature on the spectrophotometric properties of these compounds in ethanol and their reaction products with $SbCl_3$ in chloroform. The absorption maxima obtained for our compounds (Fig. 2) agree generally well with those reported in the literature, thus indicating the presence of both axerophthylidene-acetone and the C_{20}-aldehyde in our reaction. The possibility, however, of the presence of isomers of only one compound cannot be overlooked.

The treatment with acetone of pure vitamin A_1-aldehyde prepared from vitamin A_1-alcohol [1] caused no change in the retinine$_1$-molecule as judged by the U.V.-absorption curve. This is in agreement with the findings of Plack *et al.*[1]. When, however, vitamin A_1 aldehyde in concentrations equal to those present in herring roe was added to the acetone-treated residue, from which all retinine-like compounds had been removed, storage at room temperature over night resulted in it being transformed to a compound, with absorption maximum at 395 mμ. Reduction of this compound resulted in a substance with absorption maximum at 342 mμ. It thus seems that substances present in the hexane extracts from herring roe, but insoluble in acetone, are necessary for the reactions to take place which result in the formation of axerophthylidene-acetone and the C_{20}-aldehyde.

The absorption maxima for the reduction products, 340—360 mμ, suggested the presence of vitamin A_2 as one of these compounds. Haworth *et al.*[4] thus proposed a reduction of the C_{20}-aldehyde to an alcohol with a formula corresponding to the one at present accepted for vitamin A_2 [8]. Cama *et al.*[7] have discussed this possibility and pointed out that hydrogenation of the β-ionone ring in the second stage of the Oppenauer oxidation seems improbable. They discussed the possible structure for the C_{20}-compound, and suggested as one of the possibilities a *cis*-isomer of vitamin A_2 aldehyde. A reduction of this compound would result in an isomer of vitamin A_2. In this connection it should be born in mind that Haworth *et al.*[4] found the C_{20}-aldehyde to be biologically active, and Plack *et al.*[1] found approx. 40 % of the original activity of the hexane extracts in the acetone-soluble fraction, and no activity in the residue. The lack of the typical absorption maxima at 277 and 286 mμ [9,10]

Table 1. Spectrophotometric data from the literature for the Oppenauer oxidation products of vitamin A and their corresponding reduction products.

Compound	U.V. abs.max.	$SbCl_3$ abs.max.	Reference
Axerophthylidene-acetone	401	646	Batty *et al.*[3]
	395	735	Hawkins & Hunter [5]
» » reduced	354.5	712	Heilbron *et al.*[6]
	351	713	Hawkins & Hunter [5]
The C_{20}-aldehyde	401	740	Haworth *et al.*[4]
	385—395	725—735	Hawkins & Hunter [5]
» » , reduced	359		Haworth *et al.*[4]
	350—370	722	Hawkins & Hunter [5]

weighed against the possibility of the presence of vitamin A_2 after reduction of our acetone extracts. The absorption maximum for the $SbCl_3$-reaction was at 700–720 mμ, compared with 693 mμ for vitamin A_2. We may, however, emphasize that very little is known with regard to all the possible isomers of vitamin A_2 and their spectrophotometric properties. The $SbCl_3$ colour of our "aldehyde"-fraction had a maximum slightly higher than that of the reduced compound.

The present investigation has thus confirmed the findings of Plack *et al.*[1] with regard to the presence of vitamin A_1 aldehyde in herring roe. We could not find vitamin A_2 aldehyde as reported by Pollard and Bieri [2]. The effect of the acetone treatment on vitamin A_1 aldehyde when the acetone-insoluble fraction of hexane extracts of herring roe was present, establishes a case where a natural product catalyses or takes part in the reaction between vitamin A_1 aldehyde and ketone-bodies. The reactions reported, when seen in relation to the chemical studies recorded [3-5], suggest as a possible pathway for the formation *in vivo* of vitamin A_2, that it is derived from vitamin A_1 *via* the retinenes.

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Crystal Data of Nickel(II) dithiosemicarbazide-Sulphate

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K. A. Jensen [1] has described two forms of nickel(II)dithiosemicarbazide sulphate $(Ni(ThiO)_2SO_4$ which he proposed to be *cis-trans* isomers.

As very few examples of stereoismerism of nickel complexes have been definitely proved we have started an X-ray investigation in order to establish the complete structures of the two forms.

The *a* form crystallizes from water when mixing cold aqueous solutions of nickel sulphate and thiosemicarbazide. The product contains water of crystallization and the chemical analysis is consistent with the formula: $Ni(ThiO)_2SO_4$, 3 H_2O. The water is removed by heating the product to 110°C and is slowly taken up again at room temperature.

Oscillation, rotation and Weissenberg diagrams were taken of crystals of $Ni(ThiO)_2SO_4$, 3 H_2O using Cu-radiation.

The crystals are monoclinic with the following dimensions of the unit cell, unique axis b:

$$a = 6.91 \text{ Å}$$
$$b = 16.41 \text{ Å}$$
$$c = 6.32 \text{ Å}$$
$$\beta = 97°.7$$

The density of the crystal is approximately 1.84. Consequently there are two units of $Ni(ThiO)_2SO_4$, $3H_2O$ per unit cell.

The only systematic extinctions are $h\,k\,0$ when k is odd. The possible space groups are $P2_1/m$ and $P2_1$. A Patterson projektion $P(u, v)$ showed a large concentration of peaks at $v = \frac{1}{2}$. No other line exhibited extraordinary concentrations of peaks. Hence the space group $P2_1$ is established.

The β form is precipitated from hot aqueous solutions of nickel sulphate and thiosemicarbazide. It contains no water of crystallization. Its powder diagram is different from that of the *a* form. It was

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