

On the basis of Tables 1 and 2 it seems reasonable to postulate the following rules for the benzene-induced solvent shifts in the NMR-spectra of 2-hydroxy and 2-methoxy acetophenones and related chromenes:

*2-Hydroxy-acetophenones:*

1. Hydroxyl group;  $\Delta = -0.5$  to  $-0.6$  ppm (2-hydroxy-4,6-dimethoxy acetophenone shows an unexplainable high  $\Delta$ -value).
2. Acetyl-methyl group:
  - a. Free 6-position;  $\Delta = 0.5-0.6$  ppm.
  - b. Methoxyl, ether, or alkyl group in 6-position;  $\Delta = 0.2-0.3$  ppm
3. Methoxyl groups:
  - a. In 6-position;  $\Delta = 0.6-0.8$  ppm.
  - b. Others;  $\Delta = 0.4-0.6$  ppm.

When there are two *ortho* substituents to the methoxyl group the shift is decreased by 0.2–0.4 ppm, according to the findings of Scheinmann.<sup>4</sup>

*2-Methoxy-acetophenones:*

1. Acetyl-methyl group;  $\Delta = 0-0.1$  ppm.
2. Methoxyl groups:
  - a. In 2- or 6-position;  $\Delta = 0.5-0.7$  ppm.
  - b. Others;  $\Delta = 0.4-0.5$  ppm.

Where there are two *ortho* substituents to the methoxyl group the shift is decreased by 0.2–0.4 ppm according to the findings of Scheinmann.<sup>4</sup>

On the basis of these rules the structure Ia was chosen for ripariochromene. The  $\Delta$ -value for the acetyl-methyl group is 0.54 ppm which indicates a free 6-position. The lack of shift for the methoxyl group shows that both *ortho* positions are substituted.

The spectra were recorded on a Varian A-60-A spectrometer and solvents used were  $\text{CDCl}_3$  and benzene p.a. from E. Merck.

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## Developmental Changes in Lactate Dehydrogenase Isoenzyme Patterns of Rabbit Tissues

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Lactate dehydrogenase (LDH) exists in multiple forms, isoenzymes, separable by electrophoresis and by chromatography. It was postulated and later proved that the five commonly encountered LDH isoenzymes are composed of two polypeptide subunits, H and M, in various combinations of four:  $\text{LDH-1} = \text{H}_4$ ,  $\text{LDH-2} = \text{H}_3\text{M}$  etc. Various tissues possess distinct patterns; cardiac and skeletal muscle tissues have mainly  $\text{H}_4$  and  $\text{M}_4$ , respectively. The synthesis of H and M subunits is regulated by separate genes, the activities of which probably determine the LDH pattern. A given cell type can synthesize both H and M subunits, and these chains associate randomly *in vitro* to form the five isoenzymes in amounts corresponding to binomial distributions. The LDH pattern of tissues with high aerobic metabolism generally has a predominance of H subunits, whereas preferentially anaerobic tissues have a dominance of M subunits. The mechanisms for the regulation of synthesis and catabolism of H and M subunits are unknown.

Developmental changes in LDH patterns have been described in a number of species

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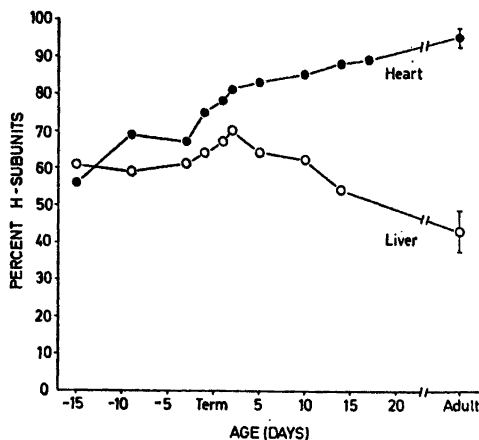


Fig. 1. Changes in percent H of the LDH pattern of rabbit heart and liver tissues during development. Each point represents the average of results on 1–3 animals except the adult values which are based on 6–9 animals. The standard deviations of adult values are indicated.

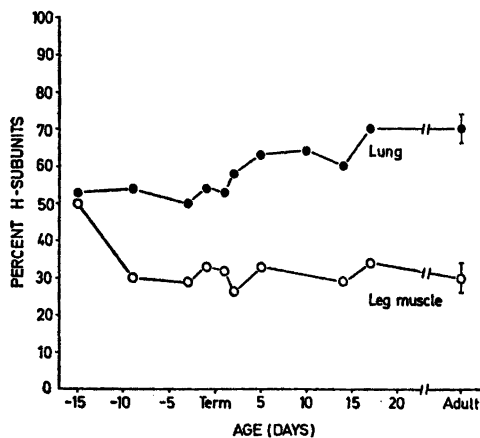


Fig. 2. Changes in percent H of the LDH pattern of rabbit lung and leg muscle tissues during development. Each point represents the average of results on 1–3 animals except the adult values which are based on 6–9 animals. The standard deviations of adult values are indicated.

among others in rabbits.<sup>1,2</sup> In view of the need for a quantitative description of developmental changes as a basis for comparative studies, this study was initiated.

*Experimental. Materials.* White rabbits (Danish Country Breed) were used. Samples were taken at different stages of prenatal development, at term and postnatally. The earliest samples were at mid-term, 15 days before term. Pregnant as well as non-pregnant rabbits were used as adults. Heart, liver, lung, and leg muscle tissues, excised at the time of sacrifice, were cut out into small pieces and washed repeatedly in cold 0.9 % sodium chloride.

*Homogenates.* The tissues were homogenized in two volumes 0.9 % NaCl in a cooled Potter-Elvehjem homogenizer and centrifuged at 15 000 g for 20 min. The supernatants were analyzed on the day of preparation.

*Electrophoresis.* The isoenzymes were separated by electrophoresis in 1.5 mm 0.5 % agarose-gel at pH = 8.3 with a voltage of 185 V.

*Analytical procedure.* The LDH activity was identified on the gel by means of a 1 % agarose-layer ("sandwich-technique"<sup>3</sup>) having the following composition: nitroblue-tetrazolium 0.13 mg/ml, phenazine methosulphate 0.01 mg/ml, nicotinamide adenine dinucleotide 0.63 mg/ml, sodium lactate 0.05 M, and sodium cyanide 0.004 M. After incubation and fixation the zymograms were dried and scanned at 576 nm in a Vitatron photometer equipped with a scanning device, automatic recorder and integrator. The percentage of H subunits was calculated on the basis of the proportional content of H subunits, i.e. 100 % for LDH-1, 75 % for LDH-2 etc. The reliability of the method as calculated from duplicate estimations was 2.8 % H subunits. Dilution of a given sample to final total LDH activities within the regions used in this study gave constant values for the percentage of H subunits.

*Results.* The percent H subunits of the LDH patterns of four rabbit tissues during pre- and postnatal development are illustrated in Figs. 1 and 2. All tissues have a common mid-term value of approximately 55 % H. With development a divergence occurs until adult values are reached at 2–3 weeks after term. For heart and lung tissues there is a gradual increase in the percentage of H subunits. The increase is most pronounced at term. Liver shows a slight increase in percentage of H around term followed by a decrease after term.

Table 1. Percent H of LDH patterns of heart and liver tissues in different animals during development.

	Species	Reference	Percent H subunits		
			Fetal	Newborn	Adult
Heart	Chicken	4	(75-100)	(75-100)	(75-100)
	Cow	2	76		86
	Rabbit	1	(70)	(75)	(95-100)
		2	48	81	>99
		*	56	76	95
	Rat	2	9-25	33	74
Liver	Chicken	4	(75-100)	(60-80)	(55-75)
	Cow	2	67		97
	Rabbit	1	(50)	(70)	(20)
		2	13	40	63
		*	61	65	44
	Rat	5	2	5	1

\* This study.

Leg muscle (quadriceps) has 50 % H subunits 15 days before term but thereafter exhibits consistently approximately 30 % H subunits during the period under investigation. In other studies it is found that muscle tissue from rabbits gradually gets richer in M subunits during development. The discrepancy might be due to the fact that different types of muscle tissues have been studied.

*Discussion.* Recently there is an increasing tendency to describe the LDH patterns in quantitative terms. Table 1 summarizes semiquantitative and quantitative data from different authors on developmental changes in 4 different species. Heart and liver tissues have been selected as most information is available for these tissues and because they represent different types of developmental changes in most of the species. The LDH pattern of heart tissues shows a gradual shift towards more H subunits except in chicken. The LDH pattern of liver tissues during development are most different in the 4 species. Table 1 further shows that the LDH isoenzyme pattern of fetal heart tissue as well as that of fetal liver tissue exhibits pronounced differences between the 4 species. On the basis of the percentage H subunits of fetal tissues in Table 1, it seems reasonable to assume that the percentage of H subunits in whole embryos are: Chicken: 75-100; cow: 65-80; rabbit: 50-70 and rat 0-10. The LDH pattern of chicken and rat thus seems to develop from pure forms (H<sub>4</sub> and

M<sub>4</sub>), whereas rabbit has an embryonic LDH pattern with nearly equal amounts of H and M subunits. Results by Fieldhouse and Masters<sup>6</sup> are in good agreement with these conclusions and have shown that sheep and pig like the cow have about 70 % H subunits and that guinea pig has about 25 % H in the embryonic pattern. Thus it is not clear whether these species develop from pure forms, from patterns with 50 % H or if the mid-term percent H values represent the embryonic values. There is no correlation between the magnitude of the embryonic percent H values of the species and the position of the species in the evolutionary table.

Alterations of the LDH pattern during development and the organ specificity may be explained by changes in the rates of synthesis of H and M subunits, changes in the catabolism of each of the isoenzymes, or both.

According to one hypothesis the synthesis of H subunits is favoured in aerobically metabolizing tissues whereas M subunits predominate in anaerobically metabolizing tissues. Studies on cells in tissue culture,<sup>7</sup> on chick embryos,<sup>7</sup> and on cultured human lymphocytes,<sup>8</sup> have shown that there is a correlation between pO<sub>2</sub> and percentage of H subunits. It is possible, however, that the regulation of the subunit synthesis is not caused by pO<sub>2</sub> *per se* but is mediated through pyruvate. Recent work<sup>9</sup> suggests that differential rates of catabolism might be important.

Another possible explanation may be changes in the intracellular distribution of LDH.<sup>10</sup>

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## Preparation of *cis*- and *trans*-*p*-Dithiane-2,3-dicarboxylic Acids and Optical Resolution of the Latter

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For certain steric correlations by the quasi-racemate method,<sup>1</sup> 2,3-disubstituted *p*-dithianes of known configuration were needed. Those reported in the literature were not suitable, either because all stereoisomers were not available, or because the absence of reactive groups prevented simple optical resolution. Moreover, their con-

figurations are not known. An unsuccessful attempt to prepare the title acids (I) from the 2,3-dibromosuccinic acids and 1,2-ethanedithiol has been reported.<sup>2</sup>

The present paper describes the preparation of *cis*- and *trans*-I via their dimethyl-esters by the reaction of 1,2-dibromoethane with the disodium salts of dimethyl *meso*- and ( $\pm$ )-2,3-dimercaptosuccinate, respectively.

As the asymmetric carbon atoms are not affected, the firmly established configurations of the starting esters<sup>3</sup> are retained in the products. This conclusion is confirmed by optical resolution of *trans*-I via its brucine and cinchonidine salts. The infrared (IR) spectra of the antipodes were identical but differed from those of both *cis*- and *trans*-I, suggesting that the latter is a true racemate.

Thorough investigations by physical means indicate that the *p*-dithiane ring prefers the staggered chair conformation,<sup>4a,b</sup> where bulky substituents are known to occupy the equatorial rather than the axial positions. On this basis, *trans*-I should be more stable than *cis*-I, as both carboxyl groups in the former may be equatorial but only one in the latter. The reverse relation was, however, indicated by almost quantitative conversion of the dimethyl ester of *trans*-I into the corresponding ester of *cis*-I in alkaline media. In order to elucidate the reasons for this unusual behaviour, an X-ray investigation of *cis*-I has been undertaken.<sup>5</sup> The relatively high melting point of *cis*-I is also remarkable.

*Experimental.* Melting and boiling points are not corrected. The melting points of the acids were determined in a preheated apparatus to minimize anhydride formation.

*Materials.* Thioacetic acid was prepared from acetic anhydride,<sup>6</sup> b.p. 87°,  $n_D^{25}$  1.4631.

*Dimethyl cis-p-dithiane-2,3-dicarboxylate.* Sodium (3.3 g, 142 mmol), dimethyl *meso*-2,3-dimercaptosuccinate (15.0 g, 71 mmol) and 1,2-dibromoethane (13.9 g, 71 mmol) were dissolved in 1000 ml abs. methanol in that order. The solution was left for 24 h at room temperature, then refluxed with stirring for another hour. The precipitate was filtered off. The methanol was removed from the filtrate by rotatory evaporation and the residue diluted with 200 ml of dry ether. The new precipitate, then the ether, were removed as before and the residual oil distilled *in vacuo*. The fraction boiling at 173–175°/9 mm was collected. It solidified within a few days and was recrystal-