Relation between relative estrogenic activity and angle of twist in substituted 4,4′-dimethoxystilbenes

Substitution on the 4,4′-dimethoxystilbene	estrogenic activity[a]	angle φ[b]	relative activity[c]
none	10	0°	<0.06 %
α-methyl	0.47	26°	~1 %
2,2′-dimethyl	5.5	28°	~0.1 %
2,2′-diethyl	5.4	30°	~0.1 %
α,2′-dimethyl	0.47	31°	~1 %
α,2-dimethyl	0.52	33°	~1 %
2,2′,6,6′-tetramethyl	0.21	45°	~3 %
α,α′-dimethyl	0.12	53°	~5 %
α,2,2′-trimethyl	0.11	60°	~5 %
α,α′-diethyl	0.006	68°	100 %
α,α′,2-trimethyl	0.0058	71°	100 %
α,α′,2,2′-tetramethyl	0.0051	90°	≥ 100 %

[a] Minimum dosis, mg. Using groups of five mice for each assay, compounds dissolved in peanut oil were injected subcutaneously. For each compound the concentration was determined that brought about cornification in vaginal smears of some – not all – mice of the test group. For exact establishment of minimum doses, more animals would be required, but we think that for the present preliminary account our method is adequate. It was verified that with 0.1 γ estradiol cornification could still be induced.

[b] Calculated from UV-data[6].

[c] Dimethylether of stilbestrol as a standard (= 100).

Biological assay was made with the Allen-Doisy test. In view of the close structural similarity of the compounds tested, we suppose that the disadvantage of the method does not influence our results. Because of difficulty in purifying the hydroxy compounds, we tested the dimethoxy compounds. As only relative potencies are compared, this method seems to be permissible.

Our results are summarized in the Table, which shows that the activity increases with the number and size of the alkyl substituents. The effect is greater with the alkyl groups in the α- than in the *o*-positions. Parallelism is obvious between the relative estrogenic activities and the angle of twist of the phenyl nuclei. It suggests that in the class of dimethoxystilbenes steric hindrance to coplanarity may be one of the factors essential to strong biological activity. The strong increase of activity for angles between 60 and 70° is remarkable. The presence of substituents of a certain form and bulkiness may also be one of the requirements for high activity. The data on the two last compounds prove that optimal activity may be obtained without ethyl groups as α-substituents.

Zusammenfassung. Für eine Reihe Methyl- bzw. Äthylsubstituierter 4,4′-Dimethoxystilbene ist die relative östrogene Wirksamkeit ermittelt worden. Dabei ergab sich eine Parallele zwischen der Aktivität und dem Grad der sterischen Hinderung, welche die Koplanarität der beiden Phenylkerne aufhebt. Maximale Wirksamkeit konnte auch mit Methyl-, nicht nur mit Äthylgruppen an der Doppelbindung erreicht werden.

W. H. LAARHOVEN, R. J. F. NIVARD, and E. HAVINGA

Laboratory of Organic Chemistry, Preclinical Institute, R.C. University, Nijmegen, and Laboratory of Organic Chemistry, The University, Leiden (The Netherlands), January 24, 1961.

Some Physico-Chemical Properties of Mouse Red Cells and Hemoglobin

Heterogeneities among adult hemoglobins from different inbred strains of mice have been demonstrated electrophoretically. By means of filter paper electrophoresis, two classes, a single-spot (Hb^1) and a diffuse type (Hb^2) have been distinguished[1]; by starch gel and starch block electrophoreses of hemoglobin from five inbred strains of mice, four distinct patterns could be observed separable by the position and number of bands[2]. The latter findings would indicate that there are probably more than the two allelic hemoglobin types that were postulated previously[3]. Although each vertebrate species was thought to have hemoglobins characteristically associated with its fetal stage and postnatal life, hemoglobins of newborn and later-stage fetuses of mice (*viz.*, C57BL/6J) were recently discovered to be indistinguishable from that of the adult upon starch gel electrophoresis; also, no significant differences of late fetal and adult mouse hemoglobin (*viz.*, CBA strain) could be detected with respect to alkali denaturation (J. BARROWMAN, personal communication). This paper reports on further methods applied to 'fetal' and adult mouse hemoglobin and red blood cells in attempts to detect specific differences.

Mice from seven inbred strains (C57BL/Ks, C57BL/6J, C57BR/cd, C58/J, DBA/1, DBA/2, BALB/c, and C3H/HeJ) and three hybrids (CAF_1, BAF_1, and BDF_1) were used. Fetal blood was derived from embryos that were at least two weeks old. Preparation of smears without placental (maternal) blood contamination from younger fetuses was technically difficult. It has been reported that hemoglobin of adult human red cells, in contradistinction to fetal hemoglobin, was readily eluted in a fixed smear by an acid citrate buffer[4-6]. In preliminary experiments with mouse red cells it was found that a slight increase of extraction time from 1 (optimal for man) to $1^1/_2$ min at a buffer-pH of 3.5 gave the most uniform results. Two stains were employed, the ferric ferricyanide reduction for free sulphydril groups and the iodination-coupled tetrazonium stain for aromatic amino acids. Human red blood cells stained by these methods also were used for purposes of comparison. Although neither of the histochemical techniques is specific for hemoglobin, under the conditions of the procedure they are satisfactory in view of the large amount (90%) of hemoglobin compared to other proteins in red blood cells. By the ferric ferricyanide reduction stain, red cells of all strains of mice, both adult and fetal, give a homogeneous deep blue color of Prussian blue prior to extraction. All strains of the original Hb^1 (C57BL/6, C57BR/cd, and C58/J), and also BALB/c (Hb^2) following acid denaturation display only a faint pale blue color, or are entirely devoid of color. A/J, DBA/1, DBA/2, and C3H/HeJ (all Hb^2) are not, or only partially extracted. In each instance adult and fetal red blood cells show equal

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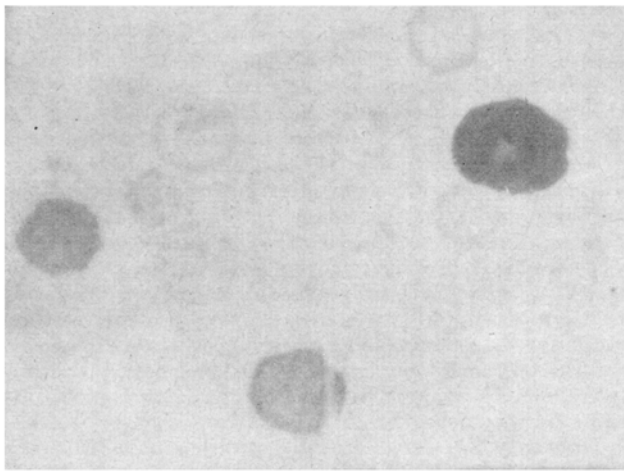

Blood smear from a 15 day old BDF_1 fetus stained by the ferric ferricyanide reduction stain for free sulphydril groups following acid extraction. Negative (extracted) red blood cells are presumably of maternal origin (C57BL/6); the unextracted (dark) cells are 'fetal' red cells staining similarly as the red cells of the father (DBA/2). × 600

acid solubility or resistance for the respective strain. Whereas human and mouse red blood cells give approximately similar intense Prussian blue stains, considerable less positive reaction is obtained in mice (tan) than man (brown) by the iodination-coupled tetrazonium stain. There were no readily perceptible differences in staining intensity among different strains of mice, or adult compared to fetal red blood cells.

Hybrid fetuses resulting from crosses of mothers having acid-extractable hemoglobin (C57BL/6 and BALB/c) and fathers with acid-resistant hemoglobin (A/J and DBA/2) contain the two types of red blood cells (Fig.). Since adult hybrids (CAF_1, BDF_1, BAF_1) have circulating red cells of only one kind, identical to their fathers in that they are not or incompletely eluted by acid, transplacental passage of maternal blood may be inferred. The presence of two kinds of hemoglobin in e.g. BDF_1 fetuses (C57BL/6 females × DBA/2 males) has also been verified by agar gel electrophoresis, showing a single spot and diffuse pattern superimposed.

Circular paper chromatography was employed using Whatman filter paper No. 1; solvents applied were 10–50% aqueous pyridine and combinations of *N*-butanol, pyridine and water. The chromatographic data reveal that the hemoglobin variants lie within a narrow range of mobility; Rf values do not differ by more than ± 0.04 units independent of the eluent used, strain of mouse, and adult or fetal origin of the hemoglobin.

Zusammenfassung. Hämoglobin und Erythrozyten von prä- und postnatal untersuchten Inzuchtmäusen verhalten sich, was verschiedene physikalisch-chemische Analysen betrifft, ähnlich. Die Existenz eines fötalen Hämoglobins konnte nicht bewiesen werden.

H. MEIER[7]

Roscoe B. Jackson Memorial Laboratory, Bar Harbor (Maine), November 22, 1961.

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Estimation of Decamethonium in Biological Fluids

In a previous work, it has been demonstrated that aqueous solutions of methonium salts, if mixed with a small amount of Nessler's reagent, develop a turbidity proportional to salts concentration. Through this very sensitive reaction, it has been possible to estimate very small amounts of methonium salts. Since this reaction is enhanced by the length of the carbon chain, it may be of a particular interest for detection of decamethonium[1] at low concentrations. On account of its strong curarizing action, C_{10} is widely employed in clinical and experimental pharmacology, and therefore its accurate estimation is very much needed in biological fluids, for which we previously postulated the use of the isotopic methods[2].

The direct application of the turbidimetric reaction to biological fluids, and specially to urine and serum, is impossible, owing to the presence of various interfering substances. It is necessary to discard these substances, without loss of C_{10}.

In this paper the results will be reported which relate to the application of this new reaction for quantitative estimation of C_{10} in biological fluids, such as blood serum and urine.

Urine procedure. To 10 ml of freshly collected normal human urine were added 100 mg of solid $HgCl_2$ and 1 ml of 2 *N* NaOH. The massive black precipitate was discarded by filtration, obtaining a slightly turbid filtrate. By addition of 2 ml of 10% HCl in glacial acetic acid, the solution becomes clear on mixing. Samples of 5 ml of the clear filtrate are collected and 0.2 ml of Nessler's reagent added. After 30 min, the turbidity was read in a Beckman spectrophotometer mod. D.U. at 580 mμ wavelength against a blank containing urine without C_{10}, similarly treated. In Figure 1, the data are given of the detection of different amounts of C_{10} added to urine.

As is shown, the urine method allows accurate determination of C_{10} concentrations between 20–200 γ/ml. It must be pointed that in the acid medium the reaction is

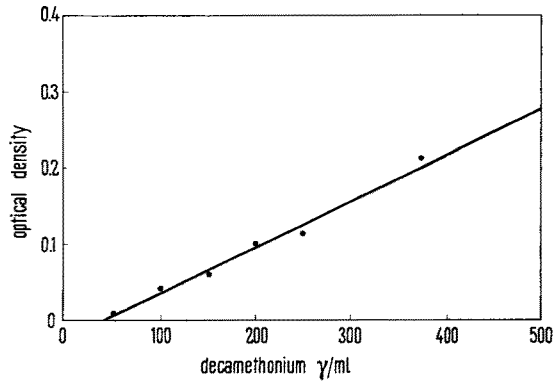

Fig. 1. Estimation by turbidity reaction of decamethonium in urine.

[1] further called C_{10}.

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