kinship of (I) and (II) to morellin[9] $C_{33}H_{38}O_7$ and moreollin[10] $C_{33}H_{40}O_8$, two known pigments from the seed coat is apparent from the chemical and biological properties[2,3,6].

The characteristic formation of analogous derivatives from (I) and (II) is, however, marked by the facile crystallisation of those of (I) presented in the Figure. (I) and (II) contain no methoxyl or free carboxylic groups. Three of the oxygen atoms in (I) are demonstrable as acidic phenol (solubility in aq. sodium carbonate), enol (formation of red copper complex analogous to isomorellin[10]) and a tertiary hydroxyl group. Two more, phenolic and carboxyl carbonyl, contribute to an α,β-unsaturated δ-lactone system. A hindered phenol, a carbonyl group para to the acidic phenolic moiety and a possible oxide ring make up for the remaining three. The presence of three double bonds is indicated by catalytic reduction of (I) and (III) to the corresponding *hexahydro-compounds* (XII) and (XIII). (I) reacts with carbonyl reagents, but the products do not seem typical. With semicarbazide and hydroxylamine, (I) forms $C_{34}H_{41}O_8N_3$, m.p. 255° (sinters at 180°), and $C_{33}H_{40}O_8N_2$, m.p. 172–174°, respectively. Similarly the *methyl ether* (III) reacts with semicarbazide and phenylhydrazine to give $C_{35}H_{43}O_8N_3$, m.p. 128–132°, and $C_{40}H_{46}O_7N_2$, m.p. 153–155°, respectively. The three phenolic groups referred to are, apparently, part of phloroglucinol recognized among the products of alkali fusion of (I) and (II) containing methylheptenol, homophthalic and isovaleric acids which are also formed by morellin[11,12]. (I) does not react with diazoaminobenzene nor couple with diazotized sulphanilic acid, thus indicating the presence of a fully substituted phloroglucinol structure as represented in the Figure. By the action of alkalies, the lactone rings in (I) and (II) are opened and the liberated acids changed to the *trans* form, *guttiferic acids* (cf. V), accompanied by a shift in the double bonds affecting the enolic group. Physico-chemical data which include ultra-violet and infrared spectra and colour reactions support the isomeric changes (vide Fig.) occurring during methylation of (I) and (III) to the *dimethyl ether* (*IV*) and of (VIII) to (IX) by method (B) *viz.*, with methyl iodide in acetone in presence of potassium carbonate, as well as during acetylation to the *monoacetyl-compound* (VI) with pyridine and acetic anhydride (method C).

Apart from formation of analogous derivatives with similar spectral characteristics, the close relationship between (I) and (II) is further suggested by oxidation with sodium hypobromite to the same *bromo-acid* m.p. 199 to 200° [λ_{max}^{EtOH} 230 mμ ($\log K = 1.625$) and 312 mμ ($\log K = 1.56$] still under investigation. These and other degradative reactions of (I) and (II) will be discussed elsewhere.

Like morellin[9,12,13], (I) and (II) are specifically active against Gram-positive bacteria and the bacteriostatic effect against *Micrococcus pyogenes* var. *aureus* (MIC 0.1–1 μg/ml) is reversed by methionine, albeit to a lesser extent. Further, they show similar cross reactions. The presence of blood serum on their antibacterial activity is less pronounced and experimental staphylococcal infections in mice are controlled by (I) and (II).

Zusammenfassung. Die Isolierung, Charakterisierung und antibiotischen Eigenschaften von α-Guttiferin (I), $C_{33}H_{38}O_8$ (Smp. 113–115°), aus den Samenhülsen und vom nahe verwandten β-Guttiferin (II), $C_{29}H_{36}O_6$ oder $C_{33-34}H_{38-40}O_8$ (Smp. 86–91°) (möglicherweise identisch mit α-Gambogasäure), aus Gummigutt, dem harzigen Sekret von *Garcinia morella*, wurden beschrieben und ihre Verwandtschaft zu Morellin und Moreollin, den beiden bekannten Pigmenten der Samenhülsen, dargelegt. Für I wurde eine Partialstruktur vorgeschlagen. Die Bildung derselben komplexen bromhaltigen Säure vom Smp. 199 bis 200° unter der Einwirkung von Natriumhypobromit auf I und II spricht ferner für deren nahe strukturelle Verwandtschaft.

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Estrogenic Activity and Steric Hindrance to Coplanarity of Alkyl Substituted 4,4′-Dimethoxystilbenes[1]

Since the pioneering work on artificial estrogens by COOK and DODDS[2], a great number of compounds have been tested for estrogenic properties[3]. Comparison of activity is difficult because of the various assay procedures that have been used. Compounds showing activity comparable to the natural female sex hormones are to be found in the classes of stilbene derivatives (stilbestrol), the diphenylethane derivatives (dienestrol and hexestrol) and among the doisynolic acids. The most active substances have in common that the molecules consist of a rather large, rigid, skeleton with, in most cases, a hydroxyl group at both ends. Possibly, there exists an optimum distance for the two hydroxyl groups[4]. From the large differences in biological activity among the α,α′-dialkyl-4,4′-dihydroxystilbenes, it is clear that also other structural features are important. OKI studied stilbene derivatives with halogeno and thiomethyl groups and concluded, from spectroscopical data, that the thickness of the molecule may be a critical factor[5]. We have measured the estrogenic properties of a series of alkyl substituted 4,4′-dimethoxystilbenes[6], in which other than steric effects are minimized.

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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.

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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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