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Note on the Serological Differentiation of Steric Isomers.

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A relationship between spatial configuration and serological specificity has been established in several instances.[1, 2, 3, 4] In recent studies it has been found that the cis-trans isomerism of maleïc and fumaric acid can be detected serologically. The immune serum used was obtained by injection of an antigen consisting of protein to which was coupled diazotized p-aminosuccinanilic acid. The reaction of this immune serum with the corresponding antigen was inhibited by succinic acid (as sodium salt) and moreover by maleïc, but not by fumaric acid. This differentiation of the cis- and trans-forms extended to the monoesters of the two acids and furthermore to the methyl substituted compounds (citraconic and mesaconic acid).

On the basis of our former results the suggestion was made[5] that in certain cases serological reactions could be used for determining spatial configuration, in a way similar to that in which enzymes have been applied to this purpose. At the same time a first example was cited dealing with the correlation between the optically active forms of malic and tartaric acids. Since no details have been given, a description of the experiments will follow.

For the tests immune sera were used specific for levo- and dextro-tartaric acids, prepared as described previously[2] and in addition an immune serum specific for levo-malic acid. This was obtained by injections of rabbits with an antigen made by coupling diazotized levo-para-aminomalanilic acid to horse serum. Levo- and dextro-rotatory para-aminomalanilic acids were prepared by reduction of the corresponding nitro-compounds.* For the preparation of these compounds the two optically active malic acids were condensed with para-nitraniline.* Which of the two possible levo- or dextro-nitranilides the substances isolated represent remains to be determined;

[1] Landsteiner K., and Van der Scheer, J., *J. Exp. Med.*, 1928, **48**, 315.
[2] Landsteiner, K., and Van der Scheer, J., *J. Exp. Med.*, 1929, **50**, 407.
[3] Avery, O., and Goebel, W. F., *J. Exp. Med.*, 1929, **50**, 521.
[4] Avery, O., Goebel, W. F., and Babers, F. H., *J. Exp. Med.*, 1932, **55**, 761.
[5] *Naturwissenschaften*, 1930, **18**, 563.
*cf. [2].

but it seems probable that the condensation would take place more readily with the carboxyl next to the hydroxyl group. For the experiment, this point is of no significance because in both levo-compounds the arrangement of the groups around the asymmetric carbon atom would correspond to one optically active form of tartranilic acid and in both dextro-compounds to the other.

Levo-para-nitromalanilic acid. The crude substance, obtained by fusion of levo-malic acid and p-nitraniline, was recrystallized twice from water and 25% alcohol. Microscopic needles. Melting point: 168-169° C. Titration: 0.127 gm. dissolved in 80% alcohol, required for neutralization 5 c.c. of N/10 NaOH. Formula $C_{10}H_{10}O_6N_2$ requires 5 c.c. A 1% solution in methyl alcohol gave at 25° a rotation of —0.77 in a 2 d.m. tube with sodium light $[\alpha]_D$ — 38.5.

Dextro-para-nitromalanilic acid. The crude substance obtained by fusion of dextro-malic acid[6] and para-nitraniline was recrystallized twice from water and 25% alcohol. Microscopic needles. Melting point: 168-169° C. Titration: 0.127 gm. dissolved in 80% alcohol, required for neutralization 4.95 c.c. N/10 NaOH. Formula $C_{10}H_{10}O_6N_2$ requires 5 c.c. A 1% solution in methyl alcohol gave at 25° C. a rotation of +0.76 in a 2 d.m. tube with sodium light; $[\alpha]_D$ + 38.

Levo-para-aminomalanilic acid. The crude material was crystallized from 8 volumes of hot water. Fine microscopic needles. The substance became dark but did not melt when heated to 230° C. Kjeldahl nitrogen determination after drying at 100° *in vacuo;* $C_{10}H_{12}O_4N_2$ calculated N 12.5%, found N 12.44%. An aqueous solution containing 0.1 gm. of the substance and 0.6 c.c. of normal HCl in a volume of 5 c.c. gave at 25° C. a rotation of —1.18 in a 2 d.m. tube; $[\alpha]_D$ — 29.5.

Dextro-para-aminomalanilic acid. The crude material was crystallized from 8 volumes of hot water. Fine microscopic needles. The substance became dark but did not melt when heated to 230° C. Kjeldahl nitrogen determination after drying at 100° *in vacuo*: $C_{10}H_{12}O_4N_2$ calculated N 12.5%, found N 12.7%. An aqueous solution containing 0.1 gm. of the substance and 0.6 c.c. of normal HCl in a volume of 5 c.c. gave at 25° C. a rotation of +1.2 in a 2 d.m. tube; $[\alpha]_D$ + 30.

An immune serum for levo-malanilic acid reacted on the levo- and not on the dextro-tartranilic antigen.

[6] Dakin, H. D., *J. Biol. Chem.*, 1924, **59**, 7.

TABLE

For a description of the technique we refer to a former publication[2]. The readings were taken after 2 hours at room temperature. The antigens were used in a dilution of 1:500 of a 5 per cent solution.

Immune sera for azo-proteins made from horse serum and	Antigens prepared from chicken serum and:			
	levo-p-amino-tartranilic acid	dextro-p-amino-tartranilic acid	levo-p-amino-malanilic acid	dextro-p-amino-malanilic acid
levo-p-amino-tartranilic acid	+±	0	+	tr
dextro-p-amino-tartranilic acid	0	+±	0	+

From the tests one may conclude that there is a correlation between the tartaric and malic acids which rotate polarized light in the same sense. Freudenberg and Brauns[7] correlated d-tartaric acid to d-malic acid by converting the former into monoacetyl-tartaric acid-dimethylester, acetyl-chloromalic acid-dimethylester, chloromalic acid and finally into d-malic acid. The fact that the same result has been arrived at by the use of immune sera seems to confirm the applicability of the serological method to questions of spatial configuration.

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Production of Experimental Endometrial Hyperplasia.*

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Endometrial hyperplasia has been known in this country since 1900, when it was described by Cullen[1]. Schroeder's[2] conclusion that endometrial hyperplasia was due to changes in the ovary resulting from a deficiency of the corpus luteum has been amply con-

[7] Freudenberg, K., and Brauns, F., *Ber. D. Chem. Ges.*, 1922, **55**, 1339.

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[1] Cullen, T. S., *Cancer of the Uterus*, Philadelphia, 1900.

[2] Schroeder, Robert. *Arch. f. Gynak.*, 1921, **98**, 81.