active of all the substances was the α,α′-dicyanostilbestrol, which exhibited the following degrees of activity:

	Dose, microg.	Reaction
α,α′-Dicyanostilbestrol	6.25	5.0
α,α′-Dicyanostilbestrol	3.12	4.5
α,α′-Dicyanostilbestrol	1.56	4.3
α,α′-Dicyanostilbestrol	0.78	3.5
Stilbestrol	0.02	5.0
Estrone	0.08	5.0

TABLE I

α,α′-DICYANOSTILBENES

	Formula	M. p., °C. (uncor.)	Analyses, % N Calcd.	Found
4,4′-Dihydroxy	$C_{16}H_{10}N_2O_2$	287	10.7	10.5
4,4′-Diacetoxy	$C_{20}H_{14}N_2O_4$	217	8.09	8.17
4,4′-Dimethoxy	$C_{18}H_{14}N_2O_2$	187	9.65	9.50
3,4,3′,4′-Dimethylenedioxy	$C_{18}H_{10}N_2O_4$	235	8.81	8.90
3,4,3′,4′-Tetramethoxy	$C_{20}H_{18}N_2O_4$	205	8.00	8.20

α,α′-DIETHYL-α,α′-DICYANODIHYDROSTILBENES

Unsubstituted	$C_{20}H_{20}N_2$	175	9.72	9.51
3,4,3′,4′-Dimethylenedioxy	$C_{22}H_{20}N_2O_4$	213	7.45	7.26
4,4′-Dinitro	$C_{20}H_{18}N_4O_4$	225	14.8	14.6
4,4′-Diamino	$C_{20}H_{22}N_4$	205	17.6	17.4
4,4′-Dihydroxy	$C_{20}H_{20}N_2O_2$	218	7.95	7.80

Acknowledgment.—The authors wish to express their appreciation to Dr. F. R. Eldred of Reed and Carnrick, Jersey City, N. J., for the numerous physiological tests performed with these substances, as well as for repeated fellowship grants.

Summary

Various substituted symmetrical cyano- and dihydrocyanostilbenes have been prepared, characterized and tested for estrogenic activity.

WASHINGTON SQUARE COLLEGE
NEW YORK, N. Y. RECEIVED JULY 11, 1942

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NEW YORK UNIVERSITY AND OF LAFAYETTE COLLEGE]

Phenylmercapto-thiazolines

BY JOSEPH B. NIEDERL AND WILLIAM F. HART

The condensation of allyl- and methallyl mustard oils with phenols yielded thiazolinephenols as shown in previous publications.[1,2] The same condensation procedure has now been applied to the reaction between allyl mustard oil and various thiophenols.

There were obtained excellent yields of hydrochlorides which analyzed correctly for the expected salts of thiazoline-thiophenols. From these, picrates were prepared which also analyzed as expected. However, when an attempt was made to prepare the free bases by neutralization of a solution of the hydrochlorides with sodium carbonate, hydrolysis occurred, one of the products being the thiophenol, and the other, 5-methyl-thiazolidone-2.

From these hydrolysis products it appears that the products of these condensations are the hydrochlorides of 5-methyl-2-phenylmercapto-thiazolines, instead of the expected thiazoline-thiophenols. The hydrochlorides of these thio-ethers are remarkably stable to acid treatment. The compounds, however, are completely hydrolyzed in dilute sodium bicarbonate solution.

(1) Niederl, Hart and Scudi, THIS JOURNAL, **58**, 707 (1936).
(2) Hart and Niederl, *ibid.*, **61**, 1145 (1939); **63**, 945 (1941).

Experimental

Condensation.—Allyl mustard oil was condensed with thiophenol, thio-*o*-cresol and thio-*m*-cresol in the manner previously described.[2] Condensation was usually complete within one week. The product was taken up in water, the acid solution extracted several times with ether, and the ether extracts discarded. The acid solution was then evaporated to dryness on a water-bath, yielding the hydrochloride in a quite pure form. The hydrochlorides were washed several times with dry acetone, and then recrystallized from 95% ethyl alcohol several times until the melting point was constant.

The picrates were made by adding a filtered, saturated picric acid solution to an equal volume of filtered aqueous solution of the hydrochloride. The resulting crystalline precipitate was washed several times with water, dried and recrystallized once from 95% ethyl alcohol.

Hydrolysis.—Twenty grams of the hydrochloride of the thio-*m*-cresol condensation product was dissolved in water, and the solution was made distinctly alkaline with sodium bicarbonate. A blue oil separated, which was removed by ether extraction and the ether extracts were in turn extracted with 10% hydrochloric acid, which removed the blue color. The ether solution was washed, dried, and the solvent removed by distillation. The residue was an almost quantitative yield of 10.5 g. of thio-*m*-cresol, which was identified by the boiling point, and by oxidation to di-*m*-cresyl di-sulfide with 3% hydrogen peroxide in acetone solution.

The hydrochloric acid extract was used to acidify the

original aqueous solution and this solution was evaporated to dryness on the steam-bath. The residue was exhaustively extracted with absolute alcohol, and the residual sodium chloride was discarded. Upon evaporation of the alcohol there was obtained an oil, which was soluble in dry acetone. This oil became crystalline on long standing in a desiccator, after which treatment with dry acetone yielded two fractions. The acetone insoluble fraction upon analysis proved to be the hydrochloride of 5-methyl-2-hydroxythiazoline. The acetone soluble fraction, which melted at 39°, proved to be its acid insoluble keto form. It was recrystallized from dry acetone and benzene.

TABLE I

Compound	M. p., °C. (uncor.)	Formula	N Analyses, % Calcd.	Found
5-Methyl-2-phenylmercapto-thiazoline				
Hydrochloride	171	$C_{10}H_{12}NS_2Cl$	5.69	5.57
Picrate	141	$C_{16}H_{14}O_7N_4S_2$	12.55	12.65
5-Methyl-2-(2′-methyl)-phenylmercapto-thiazoline				
Hydrochloride	164	$C_{11}H_{14}NS_2Cl$	5.39	5.75
Picrate	133	$C_{17}H_{16}O_7N_4S_2$	12.38	12.46
5-Methyl-2-(3′-methyl)-phenylmercapto-thiazoline				
Hydrochloride	139	$C_{11}H_{14}NS_2Cl$	5.39	5.32
Picrate	118	$C_{17}H_{16}O_7N_4S_2$	12.38	12.48
Sulfonic Acid		$C_{11}H_{13}O_3NS_2$	4.61	4.38
5-Methyl-thiazolidone-2				
Keto form[a]	39	C_4H_7ONS	11.96	12.12
Enol, hydrochloride	204	C_4H_8ONSCl	9.12	9.35

[a] Calcd.: C, 40.98; H, 6.02; S, 27.37. Found: C, 41.13; H, 6.08; S, 27.79.

Acknowledgment.—The authors desire to thank Merck and Company, Inc., Rahway, New Jersey, for a research fellowship.

Summary

Thiophenols on condensation with allyl mustard oil have been found to yield phenylmercaptothiazolines, in contrast with phenols, which yield thiazolinephenols. These heterocyclic thio-ethers do not rearrange in the presence of the usual acidic rearranging agents, but are readily hydrolyzed in alkaline solution.

WASHINGTON SQUARE COLLEGE, NEW YORK, N. Y.
LAFAYETTE COLLEGE, EASTON, PA.

RECEIVED JULY 13, 1942

[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE OF AGRICULTURE AND APPLIED SCIENCE]

The Sterols of Alfalfa Seed Oil. II. Isolation of β-Spinasterol and δ-Spinasterol

BY L. CARROLL KING AND CHARLES D. BALL

In a previous communication[1] from this Laboratory the isolation of α-spinasterol from the unsaponifiable fraction of alfalfa seed oil was reported. In addition to α-spinasterol we have now obtained two other isomeric sterols, β-spinasterol[2] and a third substance whose properties indicate that it has not before been isolated. Since this new sterol is closely related to the known α- and β-spinasterols in structure and properties we have designated it as δ-spinasterol.

In order to separate these three isomeric substances, the crude alfalfa seed oil sterols were dissolved in a large excess of acetic anhydride. On cooling this mixture, the acetates of α-spinasterol and β-spinasterol separated as flaky crystals and were filtered off while the δ-spinasteryl acetate remained for the most part in the acetic anhydride mother liquors. α-Spinasterol and β-spinasterol were separated by taking advantage of the greater solubility of the latter in 85% ethanol.

Each of the three isomeric spinasterols was isolated from two different sources—first, from Hardigan alfalfa seed oil and second, from Grim alfalfa seed oil. The crude sterols from Hardigan alfalfa seed oil consisted of about 23% α-spinasterol, 39% β-spinasterol and 6% δ-spinasterol. The amounts of the corresponding substances isolated from the crude sterols of Grim alfalfa seed oil were 17, 28 and 4.5%, respectively.

The chemical reactions and relationships of α-, β- and δ-spinasterol are summarized in Fig. 1.

δ-Spinasterol was purified by fractional crystallization from 95% ethanol and from methanol. The purest product obtained had a melting point of 142–143° and $[\alpha]^{19}D$ 6.15°. It precipitated with digitonin and gave a positive Liebermann–Burchard test.

The analytical data for δ-spinasterol and its derivatives indicated the formula $C_{29}H_{47}OH \cdot {}^1/_2H_2O$ for the sterol. On catalytic reduction of the acetate in acetic acid solution, a compound identical with α-stigmastenyl acetate[3] (I) was obtained. δ-Spinasterol is, therefore, a doubly

(1) King and Ball, THIS JOURNAL, **61**, 2910 (1939).
(2) Heyl and Larsen, *J. Pharm. Assoc.*, **22**, 510 (1933).
(3) Fernholz and Ruigh, THIS JOURNAL, **62**, 2341 (1940).