Befriedigende analytische Resultate wurden für alle erwähnten synthetischen Verbindungen erhalten[16].

[16] Wir danken den Herren Professoren T. IRIE, T. MATSUMOTO und T. MASAMUNE an dieser Universität für ihre Ermutigung zur Durchführung dieser Arbeit.
Ebenso danken wir den Herren Drs. F. E. KING und J. W. W. MORGAN für die Überlassung des Homopterocarpins.
Ein Teil der Kosten für diese Untersuchungen wurde durch das Unterrichtsministerium für wissenschaftliche Forschung gedeckt. Für diese Beihilfe möchten wir an dieser Stelle ebenfalls unseren ergebensten Dank aussprechen.

[17] Present address: University Chemical Laboratory, Cambridge (England).

Summary. The racemic form of homopterocarpin (V), a constituent of red sandal-wood, has been synthesized by a method which has previously been proposed by the authors. N.M.R. spectral studies in conjunction with the examination of models have enabled the assignment of the *cis* configuration (V) to homopterocarpin.

H. SUGINOME[17] und T. IWADARE

Chemische Abteilung der naturwissenschaftlichen Fakultät der Universität Hokkaido, Sapporo (Japan), 30. November 1961.

Some Physicochemical Properties of Anticatalase

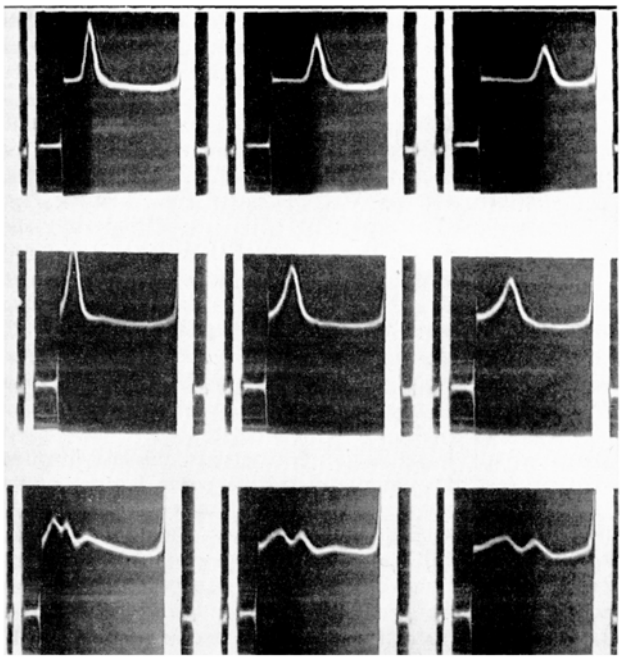
TRIA[1] reported that after injection of catalase to rabbits and consecutive test tube precipitation of the formed anticatalase from the rabbit serum by the antigen, the catalase could be denatured by a shift in pH, the denatured catalase could then be centrifuged off and the purified anticatalase would remain in the supernate. The following study was undertaken to gain more information about the chemical nature of this anticatalase.

A 0.4% lyophilized catalase (Worthington Biochemical Corp.) solution in physiological saline is administered to rabbits as follows: during the first week 0.5 ml each for four days, during the second 1.0 ml, the third 1.5 ml and the fourth 2.0 ml (also for four days). Injections after a three day interval are given subcutaneously, all others intravenously into the ear vein. 4–7 days after the last injection the rabbits are bled from the heart under nembutal anesthesia. 50 mg of crystalline catalase in suspension (Sigma Chemical Co.) are added to 100 ml of antiserum and the solution is allowed to stand at room temperature for 6–8 h. Next, it is kept for 4–7 days at approximately 3°C. The resulting precipitate is collected by centrifugation in the cold and stored while the supernate is treated again as described above by addition of catalase to a final concentration of 0.5 mg/ml etc. The combined precipitates are washed three times with equal volumes of cold physiological saline, suspended in 1.5 ml of distilled water (if 100 ml antiserum were used) and 1.5 ml 0.1 *N* HCl is added. The suspension is allowed to stand 5 min in the cold. 0.5 ml of 0.1 *M* phosphate buffer (pH 5.7) is added, followed by 1.5 ml of cold 0.1 *N* NaOH. The precipitate formed is removed by centrifugation and the supernate containing the anticatalase is dialysed for 18 h against 0.1 *M* phosphate buffer of pH 5.7. Yield: approximately 50 mg.

For the amino endgroup analysis the fluorodinitrobenzene method was used[2]. 60 mg of DNP protein were hydrolysed in a sealed tube with 10 ml of 5.7 *N* HCl at 110°C for 16 h. The hydrolysate was extracted with ether and passed through a silicic acid column. The fraction that eluted with the 17% butanol-chloroform mixture was chromatographed in toluene-chloroethanolpyridine-aqueous ammonia solvent followed by 1.5 *M* phosphate buffer (pH 6.0) in the second dimension. Agar plate precipitin tests were performed according to the method of WILSON and PRINGLE[3]. In the experiments on amino acid composition 10 mg of the dried protein were hydrolysed with 2 ml 5.7 *N* HCl for 22 h at 110°C. The resulting hydrolysate was evacuated to dryness *in vacuo* over NaOH pellets, dissolved in sodium citrate buffer pH 2.2 and analysed in the Spinco Amino-Acid Analyser[4]. Protein concentrations were determined spectrophotometrically after the $E^{1\%}_{1cm}$ (280 mμ, pH 5.7 0.1 *N* phosphate buffer) was found to be 15.2 when spectral absorption and Kjeldahl nitrogen data were correlated and a nitrogen content of 16% was assumed. Sedimentation studies on the dialysed material were performed on a Spinco Model E ultracentrifuge at an average temperature of 25°C and a speed of 59780 rpm. Pictures were taken at 16 min intervals. For the molecular weight determinations the ultracentrifuge was run at 8225 rpm. The synthetic boundary cell was used for the approach to equilibrium experiment as well as the area measurement. Data for the meniscus only were utilized.

CHARLWOOD[5] reported a $S_{20}w$ value of 6.50 for rabbit γ-globulin (in phosphate buffer, r/2 0.2 pH 6.8). Concentration-dependence was small (not more than 3–4% for a change in protein concentration of 1% w/v). Our $S^{\circ}_{w}20$ value of 6.93 for anticatalase is derived by correcting S for viscosity and density of the solvent and extrapolating to zero concentration. Using Archibald's procedure at 8210 rpm and assuming a partial specific volume of 0.735, CHARLWOOD obtained a molecular weight of 187600. Anticatalase exhibits a molecular weight of 177600. The Figure depicts ultracentrifuge sedimentation runs of catalase and anticatalase separately and combined. The shoulder indicating an impurity on the left of catalase peak becomes more apparent within the catalase-anticatalase reaction. Some catalase and some anticatalase have remained unreacted, probably because this anticatalase fraction lost activity during isolation. The question arises as to why the catalase-anticatalase peak appears so small although approximately 60% of the anticatalase material and 60% of the catalase has reacted. DEUTSCH and SEABRA[6] reported that the solubility of the catalase-anticatalase complex is much lower than that for other antigen-antibody complexes and we observed a slight turbidity in our reaction mixture probably due to undissolved precipitin. In order to rule out the possibility that the 'unreacting anticatalase' might represent another type of antibody carried as an impurity in the isolation, an

[1] E. TRIA, J. biol. Chem. *129*, 377 (1939).
[2] H. FRAENKEL-CONRAT, J. J. HARRIS, and A. LEVY, in D. GLICK, *Methods of Biochemical Analyses* (Intersciences Publishers, Inc., New York), vol. 2, p. 359.
[3] M. W. WILSON and B. H. PRINGLE, J. Immunol. *73*, 232 (1954).
[4] S. MOORE, D. R. SPACKMAN, and W. H. STEIN, Analyt. Chem. *30*, 1185 (1958).
[5] P. A. CHARLWOOD, Biochem. J. *73*, 126 (1959).
[6] H. F. DEUTSCH and A. SEABRA, J. biol. Chem. *214*, 455 (1955).



Ultracentrifuge diagrams of catalase and anticatalase before and after reaction.
The protein was dissolved in 0.1 *M* borate buffer pH 7.8. The amount of catalase or anticatalase used separately is identical to that used in the reacting system. The angles were 30° throughout, speed–41770 rpm. Pictures were taken at 16 min intervals. Upper series: Catalase. Middle series: Anticatalase. Lower series: Catalase-anticatalase mixture. In the picture on the left the most rapidly migrating peak represents the antigen-antibody complex and the intermediate peak the catalase.

Ouchterlony plate assay was performed. Only one precipitin line between the catalase and purified anticatalase could be observed.

The literature[7,8] suggests that normal rabbit γ-globulin possesses a single polypeptide chain with N-terminal alanine. Anticatalase gives one N-terminal residue which is identified as DNP-alanine by cochromatography in 1.5 *M* phosphate buffer (pH 6.0). Finally, the amino acid composition of the anticatalase is remarkably similar to that of the rabbit antibodies in general[9] (Table)[10].

Average amino acid composition of rabbit anticatalase[a]

Constituent	Constituent per 100 g protein g	Constituent	Constituent per 100 g protein g
Lysine	6.76	Glycine	5.58
Histidine	2.11	Alanine	5.39
Ammonia	1.25	Valine	9.16
Arginine	5.01	Methionine	1.30
Aspartic acid	10.07	Isoleucine	4.39
Threonine	11.78	Tyrosine	6.97
Serine	9.63	Phenylalanine	5.70
Glutamic acid	12.93	Leucine	8.50
Proline	8.91		

[a] Each value represents an average from two determinations.

Zusammenfassung. Antikatalase isoliert aus dem Katalase-Antikatalase-Präzipitin nach Denaturierung, hat einen $S^{\circ}_{20,w}$-Wert von 6,9, ein Molekulargewicht von 177600 (Archibald-Verfahren), ein $E^{1\%}_{1cm}$ (280 mμ, pH 5,7) von 15,2 und Alanin als freie endständige NH_2-Gruppe. Diese Merkmale, wie auch der Aminosäure-Gehalt der Antikatalase, sind mit den generellen Antikörpern des Kaninchens vergleichbar.

F. Friedberg

Department of Biochemistry, College of Medicine, Howard University, Washington (D.C., U.S.A.), November 24, 1961.

[7] R. R. Porter, Biochem. J. *46*, 473 (1950).
[8] M. L. McFadden and E. L. Smith, J. biol. Chem. *214*, 185 (1955).
[9] E. L. Smith, M. L. McFadden, A. Stockell, and V. Buettner-Janusch, J. biol. Chem. *214*, 197 (1955).
[10] This investigation was supported in part by a research grant, E-1953, from the National Institutes of Health, Public Health Service, and an equipment grant from the Charles F. Kettering Foundation.

Swelling of Uterus Mitochondria with Folliculin and Progesterone

Recent researches on morphology of chondrioma showed the existence of several substances capable of modifying the swelling of mitochondria, which occurs when they are suspended in isotonic sucrose, both by quickening and by inhibiting this process. As we have not found, in the abundant bibliography concerning the action of the sexual hormones on the uterus, any reference to the functional and morphological modifications of its mitochondria, we decided to investigate the effects of folliculin and progesterone, administered *in vivo*, on uterus mitochondria.

Method and Material. We prepared three groups of prepuberal female guinea-pigs, as follows: 1st group–12 control prepuberal female guinea pigs. 2nd group–12 prepuberal female guinea pigs injected intraperitoneally with 5 injections of 0.5 mg each of folliculin, every three days (Dimenformon, Organon). 3rd group–12 prepuberal female guinea-pigs injected intraperitoneally with 5 injections of 0.5 mg each of progesterone, every three days (Progestin, Organon).

Animals were killed by beheading, and then we isolated the uterus. While a part of the organ was fixed in 10% formalin solution for the histological study, 0.5 g of substance were homogenized in 0.25 *M* sucrose buffered with 0.02 *M* Tris-chloride, pH 7.4 at 4°C, by the method of Schneider[1]. 1 ml of the final concentrated suspension was equivalent to 0.25 g of fresh uterus. Changes in extinction at 520 mμ read in Beckmann model DU spectrophotometer were taken as a measure of swelling, as described by Cleland[2] and by Casu[3].

Each test tube contained 3 ml of 0.25 *M* sucrose, buffered with 0.02 *M* tris-chloride, pH 7.4; then 0.3 ml of the mito-

[1] W. C. Schneider, J. biol. Chem. *165*, 585 (1946).
[2] K. W. Cleland, Nature *170*, 497 (1952).
[3] A. Casu, Exper. *16*, 489 (1960).