presented at this laboratory, no necrophorus organisms could be found. In November, 1940, a number of ewes and rams affected with the venereal infection were made available for study. The ewes had ulcerative vulvitis and the rams had prepuce and penis lesions. In the majority of cases, the lesions were newly developed, presenting ideal material for bacteriological and virus examinations.

Aerobic and anaerobic cultures were made from the lesions of five naturally infected sheep, and six others that had been experimentally infected. Of this group of vulva, sheath and penis lesions, only the young or freshly formed ulcers were cultured. No anaerobes were recovered and none of the aerobic types were consistently present in all the lesions, with the exception of a very small Gram-negative bacillus. This organism was not pathogenic for guinea pigs or rabbits when injected intraperitoneally, and there was no evidence of an infection where pure cultures were swabbed into the scarified tissue of the vulva, prepuce or penis of experimental sheep.

Although experimental transmission of the disease was easily accomplished through the use of suspensions of the diseased tissue, a number of failures were experienced before an infective, bacteria-free filtrate was prepared. The technic by which the infective filtrates were obtained was as follows: The diseased tissue was finely ground with alundum, and then a suspension was prepared, using equal parts of beef broth (pH 8.2) and distilled water to which 5 per cent. horse serum was added. The suspension was clarified by high-speed centrifugation and the supernatant liquid was filtered. Successful filtrations were made with two virus suspensions of separate origin. The hydrogen ion concentration of the suspensions before filtration was pH 7.0 in one case and in another pH 8.2. Three infective filtrates were recovered from one suspension after passage through Berkefeld N & W candles and a 7 pound Mandler candle. The other suspension was filtered through a 3½ per cent. collodion membrane. Subcultures from these filtrates remained free of bacterial growth.

Typical lesions were produced on the prepuces of experimentally inoculated rams with each of these four filtrates. The disease was again transmitted to healthy experimental rams by prepuce inoculations with virus suspensions from two of the filtrate-produced cases. The experimental animals used in the tests and the premises on which the tests were conducted were free from infection before inoculation, as proven by uninoculated rams that were held as controls.

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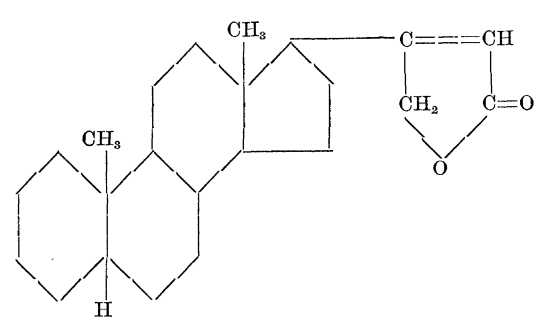
SYNTHESES OF MODEL UNSATURATED LACTONES RELATED TO THE CARDIAC AGLYCONES

SYNTHESES of β-substituted $\Delta^{\alpha,\beta}$-unsaturated γ-lactones related to the cardiac aglycones have been reported from different laboratories.[1,2] The substances described thus far represent with a high degree of certainty the lactone portion of the natural aglycones, having simple aliphatic, alicyclic and aromatic groups substituted for the cyclopentanophenanthrene part. Such compounds are not without value, and help to interpret reactions of the natural aglycones, which were difficult to explain previously. It was felt, however, that substances bearing a closer resemblance to those occurring in nature would be of interest for further study.

Of the syntheses published, that employing a carboxylic acid as starting material[3] appears to be particularly suited for the purpose in mind. From any etio acid, prepared by a Barbier-Wieland degradation of the corresponding bile acid, one proceeds to the desired lactone through the 21-acyloxy-methyl ketone, meanwhile protecting any alcoholic groups present. A similar series of reactions has recently been published by Ruzicka, Reichstein and Fuerst,[4] who converted 3,21-diacetoxy-$\Delta^{4,5}$-pregnenone-(20) into the lactone of 3,21-dihydroxy-$\Delta^{4,5;\ 20,22}$-norcholadienic acid.

We wish to report the synthesis of the lactone of 21-hydroxy-$\Delta^{20,22}$-norcholenic acid[1] in this brief note, leaving a detailed discussion for a later communication. This lactone, like digitoxigenin, thevetin and others, shows a *cis*-relationship of rings A and B as well as identical relative positions of the unsaturated lactone ring and the methyl group at C 13[5]. Etiocholanic acid through its acid chloride was converted into 21-diazo-pregnanone-(20), which with dry HCl in ether yielded 21-chloro-pregnanone-(20). This was reacted with sodium benzoate in 90 per cent. alcohol to give 21-benzoxy-pregnanone-(20), and the



[1] Elderfield, *et al.*, *Jour. Org. Chem.*, 6: 260, 1941.
[2] Ranganathan, *Current Sci.*, 9: 458, 1940.
[3] Linville and Elderfield, *Jour. Org. Chem.*, 6: 270, 1941.
[4] Ruzicka, Reichstein and Fuerst, *Helv.*, 24: 76, 1941.
[5] Jacobs and Elderfield, *Jour. Biol. Chem.*, 108: 497, 1935.

latter subjected to a Reformatsky reaction with zinc and ethyl bromo-acetate, thus effecting condensation, partial dehydration and lactonization simultaneously. The lactone[1] melts at 167–168° (corr.) and reacts positively towards Legal's and Tollens' reagents. It shows the following analytical figures: Calculated for $C_{23}H_{34}O_2$: C, 80.6; H, 10.0. Found: C, 80.4; H, 10.1.

A detailed description of this and other lactones will appear in *The Journal of Organic Chemistry.*

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE MAGNETIC PROPERTIES OF CATALASE

RECENTLY a modification of Gouy's method of measuring magnetic susceptibilities has been elaborated in this laboratory primarily for the quantitative determination of free radicals of organic dyestuffs during the process of reduction. The result is an increased sensitivity over existing methods. The method will be described in a paper now in press and may be outlined very briefly as follows.

A long cylindrical vessel with a septum in the middle, dividing it into an upper and a lower compartment, quite similar to one first used by Freed and Casper,[1] and later especially by Pauling and Coryell,[2] is suspended between the pole pieces of an electromagnet. The upper end of the suspending wire is attached to the one pan of a semi-micro balance, which is magnetically damped, very nearly critically. The pointer of the balance is equipped with a scale of 200 divisions readable through a microscope, each line corresponding to about one hundredth of a milligram. The upper compartment of the vessel is filled with a solution, or suspension, of the substance to be measured. The lower compartment is filled with the pure solvent. After switching on the magnetizing current only the maximum deflection on the microscope scale is observed, which is reached in 15 seconds. The significance of each line of deflection is previously calibrated in terms of change in magnetic susceptibility. Repeated readings allow an accuracy, according to conditions, within one or a few per cent., even when the experiment is based on a magnetic pull of, say, one fifth of a milligram. This method has been used for the measurement of the susceptibility of crystallized catalase,[3] suspended in a dilute phosphate buffer. Thus far the measurements have been made under conditions not especially favorable for weighing, *i.e.*, warm and humid summer weather, and they may be worth repeating later on under better conditions. Even so, results could be obtained which were scarcely accessible to the method of direct weighing as used by Pauling and Coryell. Since catalase has four times the molecular weight of hemoglobin, yet not more iron in one molecule than the latter, and the concentration at which a suspension—not to speak of a solution—can be obtained, is limited, the increase in sensitivity over previous methods was essential for these experiments. The result obtained so far is that the magnetic moment of catalase, per gram-atom iron, is 4.64 Bohr magnetons. The probable error, under the unfavorable conditions mentioned, is estimated to be ± 0.3. This value would be close to 4.47 as obtained by Coryell and Pauling for ferri-hemoglobin hydroxide (alkaline methemoglobin), and smaller than for ferro-hemoglobin (5.46) or ferri-hemoglobin (5.8). The magnetic experiments on catalase are being continued.

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2 L. Pauling and C. Coryell, *Proc. Nat. Acad. Sci.*, 22: 159 and 210, 1936.
3 J. B. Sumner and A. L. Dounce, *Jour. Biol. Chem.*, 125: 33, 1938; 127: 439, 1939.

GLASS ELECTRODE FOR DETERMINATION OF HYDROGEN ION ACTIVITY OF SMALL QUANTITIES OF CULTURE MEDIA

INVESTIGATIONS of changes in pH in controlled cultures necessitate means of determining the pH of relatively small quantities of fluid. It was felt that a system whereby three determinations of pH could be made from as little as 1 ml of fluid would be very advantageous. After reviewing the possibilities of several micro vessels for this work, it was decided that a relatively large durable or condenser type of glass electrode, as described by MacInnes and Belcher,[1, 2] could be used, provided it was modified in some respects and a method developed for using the modified instrument. The results have been extremely satisfactory. The instrument is very stable and rugged. It is easily cleaned without being dismantled. Furthermore, the method of sampling and determination of pH precludes errors which might arise from addition or loss of gases such as CO_2.

A glass electrode is made (Fig. 1) with the following limitations and modifications:—The Corning No.

1 D. A. MacInnes and D. Belcher, *Industrial and Engineering Chemistry*, Analytical Edition, 5: 199, 1933.
2 D. A. MacInnes and L. G. Longworth, *Transactions of the Electrochemical Society*, 71: 73, 1937.