based upon a follow-up study of crystal shapes in urines of 87 patients treated with sulfapyridine, sulfathiazole and sulfadiazine. Though varying in form from individual cases and sometimes even from the same patient on consecutive days, they were found to present forms specific for each of the three compounds investigated. Detailed data on physical and chemical analysis of these crystals will be published elsewhere. (Microphotographs of urinary crystals showing some of the typical sulfapyridine, sulfathiazole and sulfadiazine forms have been presented in several publications.[2, 3, 4, 5])

Crystallization experiments have been carried out with the urines of 51 patients receiving sulfapyridine, sulfathiazole and lately also sulfadiazine. In all specimens investigated, the *in vitro* formation of urinary crystals was attempted with acetylsulfapyridine as well as acetylsulfathiazole, regardless of which of the three compounds had been given to the patient. In recent experiments acetylsulfadiazine also was used.

For crystallization, an excess of the compound is added to the filtered and acidified urine, heated to boiling and immediately filtered. Crystals appear in the filtrate as it cools to room temperature.

It was found that urines of patients receiving sulfathiazole or sulfadiazine usually gave typical urinary crystals with both acetylsulfathiazole and acetylsulfadiazine, while with acetylsulfapyridine the forms obtained from these urines were atypical, although mostly different from crystals of the pure compound in water. Sulfapyridine urines, on the other hand, produced characteristic whetstones or arrowheads with acetylsulfapyridine, whereas the crystals formed with the acetyl products of sulfathiazole and sulfadiazine deviated more or less from their described typical appearance. In some instances sulfapyridine urines produced characteristic urinary crystals with the acetylated compounds of all 3 sulfanilamide derivatives.

Of the 51 urine specimens investigated, in 32 characteristic urinary crystals could be produced with at least one of the 3 compounds. The most typical form was always obtained with the acetyl-derivative of the drug which the patient had received. The shapes produced were identical with those shown in Fig. 1. Sixteen of the urines yielded more or less atypical crystals, while 3 gave negative results (forms as from water). These 3 urines had specific gravities between 1.010 and 1.014. In general it was observed that the production of characteristic urinary crystals may not succeed with highly diluted urines; it can, however, often be achieved with such specimens by concentrating them on the steam-bath before use for crystallization. On the other hand, urines can be depleted of their faculty to form urinary crystals by repeated supersaturation with an acetylated compound and removal of the crystals which appear on cooling. An alkaline reaction will inhibit the production of urinary crystals. The crystal-forming potency can be restored upon acidification. If urinary crystals are recrystallized from normal urine or water they assume the simple shapes which are obtained from water with the pure acetylated compounds. Urines of patients receiving sulfanilamide do not seem to possess the faculty of forming urinary crystals with the 3 compounds studied.

The artificial production of urinary crystals proves that the shapes outlined are specific for the individual compounds and are formed from their acetylated derivatives. The presence of such crystals in the urine, therefore, makes it possible to identify the particular sulfanilamide compound administered to the patient.

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A FILTERABLE VIRUS DEMONSTRATED TO BE THE INFECTIVE AGENT IN OVINE BALANO-POSTHITIS[1]

SO-CALLED venereal infection of sheep has been recognized in some sheep-raising areas of the United States and other countries for over thirty years. It is known in this country as foul sheath, sheath infection, balanitis, venereal form of lip and leg ulceration and, in Australia, it is called pizzle-rot. Filmer, in Australia, proposed the terms posthitis and balanoposthitis. Lesions are most commonly found at the prepucial orifice and on the lips of the vulva, and in the male the penis may be involved. The disease is characterized by ulceration with scab production. The more severe lesions have been noted on the prepuce and vulva. Severe sheath lesions usually result in phimosis or paraphimosis. The penis lesion is ordinarily a mild inflammation with ulceration unless accompanied by paraphimosis, which then results in a severe process with the more extensive ulceration and heavy scab formation such as is found on the prepuce.

Until late years, the disease has been classified as one of the many necrophorus infections. In a previous examination of two naturally infected rams

[1] Paper No. 155, Journal Series, Agricultural Experiment Station, Montana State College.

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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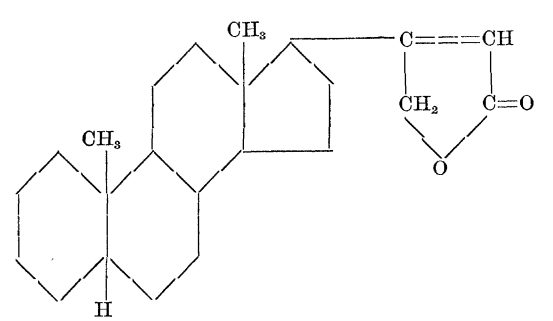
SYNTHESES OF MODEL UNSATURATED LACTONES RELATED TO THE CARDIAC AGLYCONES

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



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