TECHNICAL PAPERS

A Synthesis of Benzylpenillic Acid[1]

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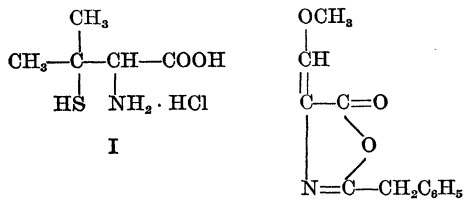
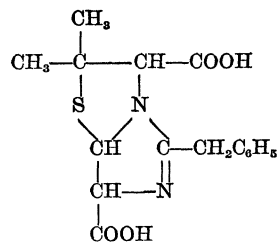
Through a condensation of D-penicillamine hydrochloride (I) with 2-benzyl-4-methoxymethylene-5(4)-oxazolone (II), and subsequent treatment of the crude condensation product, a 19% yield of D-benzylpenillic acid (D-G-penillic acid) (III) has been obtained. The compound was identical with D-benzylpenillic acid prepared from benzylpenicillin by rearrangement in aqueous solution at pH 2. The structure of D-benzylpenillic acid has already been established by synthesis (*1*).



I

II



III

The condensation of D-penicillamine hydrochloride with 2-benzyl-4-methoxymethylene-5(4)-oxazolone was carried out for 15 min at 75° in pyridine containing 5% of triethylamine. The crude condensation product was freed of pyridine, triethylamine, and triethylamine hydrochloride and was dissolved in absolute methanol. When the methanol solution was allowed to stand at 22°, needles of D-benzylpenillic acid (micro m.p., 180–182°) formed slowly. The optical rotation of the synthetic D-benzylpenillic acid was $[\alpha]_D^{20}=+471°$ (0.10% solution in methanol), while that of D-benzylpenillic acid prepared from benzylpenicillin was $[\alpha]_D^{20}=+465°$ (0.10% solution in methanol).

Starting with D-penicillamine hydrochloride, four diastereoisomers of benzylpenillic acid are possible, since there are three asymmetric carbon atoms in the benzylpenillic acid molecule. It is of considerable interest that the benzylpenillic acid isolated was the same isomer as that obtainable from natural benzylpenicillin.

When L-penicillamine hydrochloride was substituted for D-penicillamine hydrochloride in the procedure, L-benzylpenillic acid was obtained. This compound was identical with D-benzylpenillic acid in all respects, except for its opposite optical rotation, $[\alpha]_D^{21}=-476°$ (0.09% in methanol). When DL-penicillamine hydrochloride was used, optically inactive benzylpenillic acid was obtained (micro m.p., 177–179°).

As already reported in detail in the penicillin monograph (*3*), the condensation of D-penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-5(4)-oxazolone in pyridine (containing no triethylamine) yields a small amount of antibiotic activity. A great deal of evidence indicates almost beyond doubt that this synthetical activity is due to benzylpenicillin.[2]

It has also been reported (*3*) that the concentration of acid in the pyridine solution greatly affects the formation of activity. On further investigation of this effect, we have found that an increase in the concentration of acid increases the rate of formation of activity but does not greatly affect the amount of activity formed. The addition of triethylamine to the pyridine solution, on the other hand, apparently interrupts the reaction at an intermediate stage. The crude product of such a condensation of D-penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-5(4)-oxazolone in pyridine solution containing 5% triethylamine possesses no antibiotic activity. Its ultraviolet absorption spectrum has a strong maximum at 3,200 A ($E_M = 26{,}000$–$28{,}000$; calculated for a molecular weight of 334). The usual amount of activity can be produced, however, by heating this crude condensation product in pyridine solution containing pyridine hydrochloride (*2*).

In the course of fractionation studies on the crude product obtained by the condensation of penicillamine and the oxazolone in the presence of triethylamine, the formation of the D-benzylpenillic acid was encountered. The compound was obtained in a 5% yield from a rather concentrated solution of the crude condensation product in a mixture of hexane, chloroform, ethanol, and water which had been allowed to stand overnight at 22°. Sub-

[1] This manuscript, submitted on March 29, 1946, for publication to follow the appearance of the projected monograph, *The chemistry of penicillin,* has been released for publication by the Editorial Committee of the monograph.

[2] Since this paper was submitted for publication, benzylpenicillin has been isolated from this reaction mixture (V. du Vigneaud, F. H, Carpenter, R. W. Holley, A. H. Livermore, and J. R. Rachele. *Science,* 1946, **104**, 431).

sequently it was found that a 10% yield could be obtained in 65% ethanol–35% water, and the yield was increased to 15% in absolute ethanol and 19% in absolute methanol.

In a typical experiment, 60 mg of D-penicillamine hydrochloride and 66 mg of 2-benzyl-4-methoxymethylene-5(4)-oxazolone were condensed in 11.4 cc of pyridine and 0.6 cc of triethylamine at 75° for 15 min. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 10 cc of chloroform. The chloroform solution was extracted with 10 cc of cold 2 *M* pH 1.6 phosphate buffer and with 10 cc of 1.5 *M* pH 5.2 phosphate buffer, and was dried over anhydrous sodium sulfate. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 2.0 cc of 95% ethanol. Crystals of D-benzylpenillic acid formed over a period of 24 hrs, and during this time the absorption at 3,200 A gradually dropped. After 44 hrs, 14.5 mg of D-benzylpenillic acid were obtained.

The identity of the compound was established by its melting point, mixed melting point with authentic D-benzylpenillic acid, ultraviolet absorption spectrum, optical rotation, and conversion to dimethyl D-benzylpenillate (*1*).

References

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Atrophy of Ovaries Transplanted to the Spleen in Unilaterally Castrated Rats; Proliferative Changes Following Subsequent Removal of Intact Ovary[1]

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It has been shown that the transplantation of an ovary into the spleen of a castrate female rat leads to the development of a luteoma in that ovary after a period of approximately 5 months. If the transplant is permitted to grow for 10 months or more, a granulosa cell tumor supervenes (*1, 2*). The major factor concerned is the hormonal imbalance that results from inactivation by the liver of the estrogenic and other hormones elaborated by the transplanted ovary. The histogenesis of the tumor has been described (*2*). The important changes are the continuous formation of new primordial follicles, transformation of the follicles into corpora lutea, and growth of the corpora lutea with the formation of a large encapsulated luteoma in which granulosa cell nests arise (*2*). The appearance of these tumors after intrasplenic ovarian transplants in mice, guinea pigs, and rabbits has been described (*3, 5, 6, 7*). Confirmation of the growth in rats of the ovarian transplant (*4*) and formation of the tumors (*7*) has been published.

During the original experiments it had been found that if adhesions formed between the spleen and the systemic circulation so that the ovarian hormones by-passed the liver, the growth of the transplant was greatly retarded or would not take place. It was also noted that a pellet of estrogen placed in the subcutaneous tissue had the same effect on the transplanted ovary.

In the present experiments rats of the Long-Evans strain were used. In the first group there were 3 immature and 3 mature females. In the case of each animal one ovary was removed, cleaned of adherent structures, and placed in a pocket under the capsule of the spleen. The other ovary was not disturbed. One animal was sacrificed at each of the following days after transplantation: 24, 55, 90, 130, 150, and 246. In all animals the ovary *in situ* had undergone the usual compensatory hypertrophy that takes place after unilateral ovariectomy. The gross and histologic changes were the same in the immature and mature animals. The transplant in the spleen was much smaller than the original ovary; its position was indicated by the scar on the capsule of the spleen. Histologic preparations were made in the customary manner, and serial sections were studied. The changes were uniform except that in the youngest transplant, after 24 days, there were many small, active follicles with intact ova. No corpora lutea were present. The interstitial tissue was distorted by fibrous tissue. In all of the remaining animals the histologic structure was characterized by extremely few follicles that were relatively small in size. If ova remained, they appeared degenerated. There were no newly formed corpora lutea, and those that had been present in the mature ovaries had disappeared. The interstitial tissue was increased. In the animal examined after 246 days the degree of atrophy was severe; only two minute follicles were noted in a dense, fibrous interstitial tissue.

The second series of 4 immature animals was prepared by transplanting the left ovary into the spleen. After intervals of 63, 91, 91, and 91 days the hypertrophied right ovary was removed. After additional periods of 69, 97, 122, and 143 days the animals were sacrificed. Gross examination showed enlargement of the transplant. In the 3 oldest it was approximately 1 cm in diameter. Each uterus was atrophic, and the vaginal smears showed mainly leucocytes. Serial sections of the transplants were prepared. In the 69-day-old specimen the ovary was replaced by congeries of closely packed, large corpora lutea. Each corpus was quite distinctly outlined, and usually contained a small, central fibrous core. Scattered throughout were a few developing follicles. After 97 and 122 days the pattern was similar except that a rare fol-

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