CHEMISTRY

==========

1960

SovietRxiv

Abstract

Full Text

CHEMISTRY

A. S. KHOKHLOV, M. A. PANINA, and A. V. UVAROV*

PREPARATION AND PROPERTIES OF PENICILLIN NITRILES

(Presented by Academician M. M. Shemyakin, 27 VI 1960)

One of the important groups of antibiotics is constituted by the penicillins, which are heterocyclic acids of the general formula (I)

$$\begin{array}{c} \mathrm{RCONH{-}CH{-}HC\quad S\quad C(CH_3)_2} \\ \qquad\qquad\quad | \qquad\qquad | \\ \mathrm{CO{-}N{-}CH{-}R'} \end{array} \qquad \text{(I)}$$

$$\mathrm{R = C_6H_5CH_2,\ C_6H_5OCH_2;\quad R' = COOH}$$

They have been studied in detail from the biological and chemical standpoints; however, a number of questions concerning the dependence of their biological action on structure have not yet been investigated. In particular, there is little information concerning the significance of the free carboxyl group and the biological activity of compounds in which it is replaced by other groupings. It has been established that methyl, ethyl, and other esters (I, $\mathrm{R' = COOAlk}$) are inactive *in vitro*, while their activity in the organisms of some animals is associated with saponification to the free acids. The NN-diethylaminoethyl ester of benzylpenicillin (I, $\mathrm{R = C_6H_5CH_2}$, $\mathrm{R' = COOCH_2CH_2N(C_2H_5)_2}$) and analogous compounds are saponified most readily, as a result of which they find application in medicine. The unsubstituted amides of benzylpenicillin (I, $\mathrm{R = C_6H_5CH_2}$, $\mathrm{R' = CONH_2}$) and phenoxymethylpenicillin (I, $\mathrm{R = C_6H_5OCH_2}$, $\mathrm{R' = CONH_2}$), as well as the phenylhydrazide of phenoxymethylpenicillin (I, $\mathrm{R = C_6H_5OCH_2}$, $\mathrm{R' = CONHNHC_6H_5}$), possess slight antibacterial activity. By contrast, various substituted amides of penicillins are completely devoid of antibacterial properties (I, $\mathrm{R = C_6H_5CH_3}$, $\mathrm{C_6H_5OCH_2}$, $\mathrm{R' = CONHCH_2C_6H_5}$, $\mathrm{CONHC_7H_{15}}$, etc.), including carboxyanilidephenoxymethylpenicillin (I, $\mathrm{R = C_6H_5OCH_2}$, $\mathrm{R' = CONHC_6H_4COOH}$-*n*), which contains a free carboxyl group.

The present work describes the preparation and certain properties of the previously unknown nitriles of benzylpenicillin (I, $\mathrm{R = C_6H_5CH_2}$, $\mathrm{R' = CN}$) and phenoxymethylpenicillin (I, $\mathrm{R = C_6H_5OCH_2}$, $\mathrm{R' = CN}$). The corresponding

amides (I, R = $C_6H_5CH_2$, $C_6H_5OCH_3$; R′ = $CONH_2$), which had been obtained by previously described methods ([1,2]), were chosen as starting products for their preparation. Further study of the phenoxymethylpenicillin amide obtained showed that it is capable of forming stable solvates with 1 mole of ethanol and 1 mole of dioxane; these are crystalline substances with melting points, respectively, 98° and 133—135°.

Found, %:	C 55.15; H 6.78; N 10.62
$C_{16}H_{19}N_3O_4S \cdot C_2H_6O$. Calculated, %:	C 54.98; H 6.39; N 10.6

Found, %:	C 54.84; H 6.46; N 9.62
$C_{16}H_{19}N_3O_4S \cdot C_4H_8O_2$. Calculated, %:	C 54.9; H 6.21; N 9.6

* A. V. Uvarov carried out the determination of the IR spectra of the substances described in this communication.

On recrystallization from chlorobenzene, phenoxymethylpenicillin amide separates without solvent in the form of prisms with m.p. 158–159°. Its biological activity is 100 units/mg.

$C_{16}H_{19}O_4N_3S$.	Found, %:	C 54.93; 55.19; H 5.52; 5.78; N 12.12; 11.82
	Calculated, %:	C 54.98; H 5.48; N 12.03

Benzylpenicillin amide behaves similarly; it also forms a solvate with 1 mole of dioxane, m.p. 112–114°. On recrystallization from chlorobenzene, benzylpenicillin amide separates without solvent in the form of rhombs, m.p. 160–161°. As is known ([3,4]), phenoxymethylpenicillin is relatively stable in an acidic medium. Therefore, the first attempts to obtain the nitrile were undertaken starting from phenoxymethylpenicillin amide by dehydrating it with P_2O_5, $(CH_3CO)_2O$, and $Mg(ClO_4)_2$ in various solvents. Depending on the nature of the reagent, the reaction time varied from 0.5 to 24 h. When the reaction was carried out in the presence of P_2O_5 and $(CH_3CO)_2O$, profound changes of the amide occurred, as indicated by the appearance of an intense coloration of the reaction mixture and loss of the ability to undergo iodometric titration under the conditions used for determining penicillins.

Fig. 1. Effect of temperature (I) and reaction time (II) on the yield of phenoxymethylpenicillin nitrile

The use of $Mg(ClO_4)_2$ likewise did not give positive results, since even on prolonged heating in chlorobenzene with this reagent the amide for the most part remained unchanged. Therefore, the dehydration of amides with *p*-toluenesulfonyl chloride in the presence of pyridine was begun ([5]). In this way, in particular, the nitriles of 7-chlortetracycline and 5-oxytetracycline were obtained ([6]). However, the procedure used in the latter case proved unsuitable for obtaining penicillin

Fig. 2. IR spectra

Figure 1: Fig. 2. IR spectra

nitriles, since penicillin amides do not enter into reaction under these conditions. In order to determine the optimum conditions, the dehydration reaction was carried out at temperatures from 3 to 70°.

From Fig. 1, *I*, it is seen that with increasing temperature the yield of nitrile increases. Therefore, subsequently the reaction was carried out at 67-69°. To determine the optimum reaction time, it was conducted under the indicated temperature conditions (ratio of amide, *p*-toluenesulfonyl chloride, and pyridine $1 : 3.75 : 20.2$) from 5 to 70 min. As is seen from Fig. 1, *II*, the best yield of nitrile was obtained with a reaction time of 30 min.

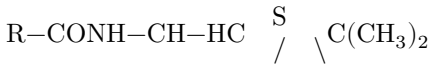
Thus, the optimum conditions for obtaining phenoxymethylpenicillin nitrile are a reaction time of 30 min at 67-69°. Benzylpenicillin nitrile was obtained by the same procedure, but under milder conditions (see Experimental section). The compounds obtained exhibit weak biological activity: benzylpenicillin nitrile, 70 units/mg; phenoxymethylpenicillin nitrile, 10 units/mg. The nitriles obtained under these conditions retained the thiazolidine-β-lactam bicyclic system characteristic of penicillin, as evidenced by the analytical data and by their ability to undergo iodometric titration under the conditions used for determining penicillin amides ([2]). For more rigorous proof of the presence of a nitrile group in the compounds obtained, their IR spectra were studied and compared with the spectra of the corresponding amides (Fig. 2). The spectra were recorded on an IKS-14 double-beam spectrometer with a NaCl prism. All samples were examined in the crystalline state as pastes with Vaseline oil.

In the spectra shown, a very intense band is observed at a frequency of the order of 1790-1802 cm^{-1}, which, according to the literature data, is characteristic of the carbonyl of the β-lactam ring ([7,8]).

In the spectra of the nitriles (Fig. 2, 1, 2), maxima are visible at a frequency of 2240-2242 cm^{-1}. In the amides this absorption band is absent. It is known ([8]) that the detection of this band in the spectrum serves as sufficient grounds for asserting the presence of a nitrile group in the compound. Its low intensity is due to the influence of oxygen-containing groups, in particular the carbonyl of the β-lactam ring of the molecule.

Fig. 2. IR spectra of benzylpenicillin nitrile (1), phenoxymethylpenicillin nitrile (2), benzylpenicillonic acid nitrile (3), phenoxymethylpenicillonic acid nitrile (4), benzylpenicillin amide (5), and phenoxymethylpenicillin amide (6)

For more definite identification of the nitrile group, the nitriles obtained were converted, respectively, into β-nitrile of benzylpenicillonic acid and β-nitrile of phenoxypenicillonic acid (II, $R = C_6H_5CH_2, C_6H_5OCH_2$).



$$\text{COOH} \quad \text{NH}-\text{CH}-\text{CN} \qquad \text{(II)}$$

In the spectra of these compounds (see Fig. 2, 3, 4), the absorption bands of the nitrile group are revealed more clearly.

Experimental part

The dehydration reaction is carried out in anhydrous pyridine. To a solution of the amide in pyridine, with stirring, a freshly prepared solution of *p*-toluenesulfonyl chloride in pyridine is slowly added. The temperature of the reaction is then raised to the specified value, and the reaction mixture is stirred for 20-30 min. After completion of the reaction, the mass is poured into ice water. The precipitated solid is filtered off, washed with water, dried, and recrystallized from the appropriate solvent.

Reaction of phenoxymethylpenicillin amide with *p*-toluenesulfonyl chloride in pyridine. To 1 g of amide

(0.00286 mole) in 3.0 ml of pyridine, 2.04 g (0.011 mole) of *p*-toluenesulfonyl chloride in 1.7 ml of pyridine is added at 66-68° (total pyridine 4.7 ml, 0.0576 mole). The mixture is then stirred at this temperature for 30 min, after which the reaction mixture is cooled to 20° and poured into 18 ml of ice water. The precipitate that separates is filtered off, thoroughly washed with water, and recrystallized from ethanol. Yield 0.45 g (47%), m.p. 97-98°. Crystal form: needles; biological activity 10 units/mg.

Found, %: C 58.22; H 5.5; N 12.8
$C_{16}H_{17}N_3O_3S$. Calculated, %: C 58.17; H 4.88; N 12.71

Reaction of benzylpenicillin amide with *p*-toluenesulfonyl chloride in pyridine. Benzylpenicillin nitrile was obtained by the procedure described above, with the same ratio of components. Reaction temperature 62-63°, duration 20 min. After recrystallization from ethanol, the yield of nitrile was 20%, m.p. 142-143°; crystal form: elongated prisms; biological activity 70 units/mg.

Found, %: C 60.90; H 5.57; N 13.33
$C_{16}H_{17}N_3O_2S$. Calculated, %: C 60.94; H 5.42; N 13.31

Preparation of phenoxymethylpenicilloic acid nitrile. 0.1 g of phenoxymethylpenicillin nitrile is treated with 12 ml of 0.05 *N* NaOH, the pH of the solution being 12.0. The suspension is left for 3 hours at room temperature, after which the reaction mixture is filtered, the solution is acidified to pH 1.8-2.0, and left for 24 hours in a refrigerator. The precipitate that separates is filtered off, washed with water, dried, and recrystallized from methanol.

Yield 0.062 g (59%), m.p. 68°. Benzylpenicilloic acid nitrile was obtained by an analogous method.

The authors express their gratitude to E. N. Druzhinina for determining the biological activity of the compounds described and to F. M. Meller for carrying out the microanalysis.

All-Union Scientific Research
Institute of Antibiotics

Received
18 VI 1960

REFERENCES

1. D. E. Cooper, S. B. Binkley, J. Am. Chem. Soc., **70**, 3966 (1948).
2. A. S. Khokhlov, E. M. Kleiner, L. B. Sinyavina, *Antibiotiki*, **3**, 5, 44 (1958).
3. A. P. Kondrat' eva, B. P. Bruns, *Med. prom. SSSR*, **12**, 30 (1957).
4. E. Brandl, H. Margreiter, *Österr. Chem. Ztg.*, **55**, 1-2, 11 (1954).
5. C. R. Stephens, E. J. Bianco, F. J. Pilgrim, J. Am. Chem. Soc., **77**, 1701 (1955).
6. C. R. Stephens, L. H. Conover et al., J. Am. Chem. Soc., **76**, 3573 (1954).
7. *The Chemistry of Penicillin*, Princeton, New Jersey, 1948.
8. L. Bellamy, *Infrared Spectra of Molecules*, IL, 1953.

Note: Figure translations are in progress. See original paper for figures.