studies will serve in research of utilization of this toxic substance.

SUMMARY

Biological activities of a new toxic substance, Teleocidin, produced by a *Streptomyces* were examined. Among the toxic antibiotics produced by *Streptomyces*, 9 antibiotics were also tested for their toxicity toward *Oryzias latipes*, *Daphnia magna* and *Bugula neritina* larvae, and Antimycin A was found to show equal or more severe toxicity than Teleocidin.

Teleocidin has shown certain inhibitory actions to *Ascaris suilla* and *Turbatrix aceti* and also has severe toxicity and skin irritative effects on mammals.

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Studies on the Reduction of Terpenes with Sodium in Aqueous Ammonia

Part XI. On the Reduction of Isopulegone*

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On the reduction of isopulegone with sodium in aqueous ammonia, it was found that the main product was isopulegol. This fact indicates that the double bond not in conjugation with carbonyl group is not attacked.

In the previous papers, it has been shown that saturated ketones ((-)-menthone¹⁾, (-)-isomenthone²⁾, (±)-menthone³⁾, (±)-isomenthone³⁾,

inverted menthone⁴⁾), α,β -unsaturated ketones ((-)-carvone⁵⁾, (+)-pulegone⁶⁾, (±)-piperitone⁷⁾) and cross conjugated ketone (piperitenone⁸⁾)

* For Part X see This Bulletin, **24**, 601 (1960).

1) H. Ueda, Y. Shibahara and S. Shimizu, This Bulletin, **23**, 376 (1959).

2) H. Ueda, *ibid.*, **24**, 595 (1960).

3) H. Ueda, *ibid.*, **24**, 505 (1960).

4) H. Ueda and S. Shimizu, *ibid.*, **23**, 380 (1959).

5) H. Ueda and S. Shimizu, *ibid.*, **23**, 524 (1959).

6) H. Ueda and S. Shimizu, *ibid.*, **24**, 92 (1960).

7) H. Ueda, *ibid.*, **24**, 598 (1960).

8) H. Ueda, *ibid.*, **24**, 601 (1960).

yielded saturated alcohols by the treatment with sodium in aqueous ammonia, i.e., a novel method of reduction employed in this series.

The present investigation was undertaken to determine the composition of the reduction product of isopulegone (β,γ -unsaturated ketone), on the same reduction as described above.

In the reduction of carvone, the carbonyl group and double bond conjugated with the carbonyl group in carvone were reduced, but the unconjugated double bond was not affected, dihydrocarveol being produced⁵¹. In view of this result, it is presumed that isopulegone would be reduced with sodium in aqueous ammonia to give isopulegol.

On the other hand, from the fact that isopulegone is easily converted to pulegone by the treatment with sodium-ethoxide or baryta⁹⁾, it is also possible to suppose that isopulegone would be converted by alkali in reaction medium to pulegone, which would be reduced to menthol, as reported previously⁶⁾.

The experiment described below was undertaken in order to make a choice between these alternative presumptions.

The experiment showed that the reduction product of isopulegone consisted of 90% of unsaturated alcohol and 10% of menthol. The unsaturated alcohol was characterised mainly as (–)-isopulegol by the bromide of its 3,5-dinitrobenzoate. Catalytic hydrogenation of the reduction product gave (–)-menthol predominantly.

This showed that the majority (90%) of isopulegone is reduced to isopulegol by the treatment with sodium in aqueous ammonia, though a minor amount (10%) give menthol through pulegone.

EXPERIMENTAL

All melting and boiling points were uncorrected. Ultra violet spectra were determined at Prof. Shimizu's Laboratory, Shinshu University.

Isopulegone:

9) J. L. Simonsen, "The Terpenes" Cambridge Univ. Press, 1953, Vol. I, p. 389.

Isopulegol, prepared from citronella¹⁰⁾, b.p. 69.0°/6.5 mm, $d_4^{18.5}$ 0.9148, $n_D^{18.5}$ 1.4731, MR 47.31 (Calcd. 47.24), $[\alpha]_D^{18.5}$ –1.0° (homog.), was oxidised by sodium dichromate in acetic acid at 60°. The oxidation product was a colorless oil, which was confirmed to be composed mainly of isopulegone containing a trace amount of pulegone by assay of its ultraviolet spectrum (pure pulegone had $\lambda_{\max}^{\text{MeOH}}$ 253 m μ , $E_{\max}^{\text{MeOH}}$ 7500¹¹⁾); Yield 74.1%, b.p. 72.5°/7 mm, d_4^{20} 0.9158, n_D^{20} 1.4696, MR 46.34 (Calcd. 45.72), $[\alpha]_D^{20}$ –3.7° (homog.), $\lambda_{\max}^{\text{MeOH}}$ 253 m μ , $E_{\max}^{\text{MeOH}}$ 137.

Reduction of isopulegone:

Isopulegone (10.0 g) was reduced with sodium (7.5 g) in aqueous ammonia and *n*-hexane (reaction temp. 0~3°), in the same way as previously reported¹⁾. The reduction product (9.8 g, Yield 96.7%) was a colorless oil, and did not crystallise on cooling (–22°); b.p. 73~75°/7 mm, d_4^{20} 0.9028, n_D^{20} 1.4698, MR 47.71 (Calcd. 47.24), $[\alpha]_D^{20}$ –12.0° (homog.), $\lambda_{\max}^{\text{MeOH}}$ 268 m μ , $E_{\max}^{\text{MeOH}}$ 4.

Identification of (–)-isopulegol:

The reduction product (2.07 g) gave crude 3,5-dinitrobenzoate, m.p. 85~101°, in a quantitative yield. Two crystallisations of its crude ester from *n*-hexane gave pale yellow needles (2.62 g, Yield 56.1% based on the reduction product), m.p. 103~109°, iodine-number 62.80. The iodine-number was determined by Rosemund-Kuhnhenh's method, using pyridine-bromine-sulphate⁵⁾, and it indicated that its 3,5-dinitrobenzoate fraction contained 86.2% of unsaturated ester. One crystallisation from *n*-hexane of the crude bromide (310 mg), recovered from titrated solution of iodine-number determination of its 3,5-dinitrobenzoate (201 mg), gave (–)-dibromoisopulegyl 3,5-dinitrobenzoate (178 mg, Yield 70.5% for theor.), m.p. 110°, undepressed by admixture with an authentic sample. The authentic (–)-dibromoisopulegyl 3,5-dinitrobenzoate, m.p. 110°, was prepared from (–)-isopulegyl 3,5-dinitrobenzoate, m.p. 104~104.5°¹⁰⁾, in the same way as described above.

Catalytic hydrogenation of the reduction product:

The reduction product (3.48 g) was hydrogenated over a platinum oxide catalyst (50 mg) in acetic acid. It absorbed 489 ml (21°C) of hydrogen, equivalent to 89.8% of the theory on the basis of isopulegol, and gave menthol mixture 3.13 g, Yield 88.7% for theor.); b.p. 80~81°/6 mm, m.p. 22~23.5°, d_4^{25} 0.8893, n_D^{25} 1.4580, MR 47.94 (Calcd. 47.71), $[\alpha]_D^{25}$ –29.9° (homog.). The menthol mixture (496 mg) gave (–)-menthyl 3,5-dinitrobenzoate (705 mg, Yield 63.4%), m.p. 152~153°, undepressed.

10) H. Ueda and S. Shimizu, This Bulletin, **24**, 402 (1960).

11) S. Shimizu, *J. Shinshu Univ. Japan*, No. 4, 73 (1954).

by admixture with an authentic sample¹²⁾.

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¹²⁾ H. Ueda and T. Mitsui, *J. Agr. Chem. Soc. Japan*, **28**, 945 (1954).

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Molecular Complexes of Tetracycline Acid Salts

Part I. The Preparations and Properties of Tetracyclinium Salt-Oxalic Acid Complexes*

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A series of studies on the crystalline molecular complexes of tetracycline groups have been carried out in this laboratory, and the present paper deals with the compositions of the oxalic acid complexes of various tetracycline acid salts, which were examined by X-ray powder diffraction methods, infrared spectra, and chemical analyses. Insertions of salt anions and crystal water in the crystal lattice of the complexes, and the important role of the latter for crystallization are indicated.

Broad-spectrum antibiotic tetracycline (TC), which was first obtained from catalytic reduction of 7-chlorotetracycline¹⁾ and later by the direct fermentation of a *Streptomyces*²⁾, shows interesting physico-chemical behaviors different from other acid and basic antibiotics.

Some properties of TC are attributable to its amphoteric character including unusual insolubility

in neutral aqueous solution. Due to these properties, TC hydrochloride is generally used clinically and its alkaline earth metal salts are usually applied for the precipitation of TC from the fermentation broths owing to the sparing solubility of these salts. Aromatic sulfonic acid salts of TC have also been reported³⁾.

Furthermore, the strong polarity and the polyfunctional character of TC may suggest the formation of complexes having different solubilities. Indeed, many complexes of tetracyclines have been described in the literatures as follows: Calcium chloride complex of 5-

* S. Inouye and H. Ogawa, The paper presented at the Kanto Local Meeting of the Agricultural Chemical Society of Japan, February 27, 1960.

1) J. H. Boothe, J. Morton, J. P. Petisi, R. G. Wilkins and J. H. Williams, *J. Am. Chem. Soc.*, **75**, 4621 (1953); W. T. Moreland, A. R. English, C. R. Stephens and F. J. Pilgrim, *J. Am. Chem. Soc.*, **75**, 4622 (1953).

2) H. Ogawa and T. Ito, *J. Agr. Chem. Soc. Japan*, **30**, 123 (1956); P. P. Minieri, H. Sokol and M. C. Firman, U. S. Patent No. 2,734,018 (1956).

3) F. A. Marchetti and G. Casini, *Ricerca. scie.*, **26**, 3321 (1956).