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ERYTHROMYCYLAMINE

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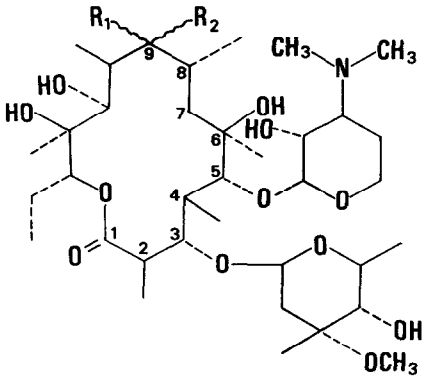
Recent reports[1,2,3] of the preparation and properties of erythromycin oxime and "9-amino-3-O-cladinosyl-5-O-desosaminyl-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethylpentadecane-13-olide," also referred to as "erythromycylamine"[3], prompt us to relate our findings which differ in several instances.

In 1956 an antibacterially active substance designated erythromycylamine was obtained in low yields in our laboratory from the erythromycin-hydrazine adduct (II)[4] and its N'-isopropylidene derivative (III) by catalytic reduction (PtO_2) in glacial acetic acid solution. Shortly thereafter, erythromycylamine (Va), judged to be a single entity by the physical methods available at that time, was also obtained in improved yields by catalytic reduction of erythromycin oxime (IV). The current re-examination of these conversions is summarized in this report.



I R_1R_2 = =O
II R_1R_2 = =N–NH_2
III R_1R_2 = =N–N=C(CH$_3$)$_2$
IV R_1R_2 = =N–OH
IVs R_1R_2 = =NOH, HCl salt
Va R_1= NH_2; R_2= H
Vb R_1 = H; R_2= NH_2

When erythromycin (I) is treated with hydroxylamine in dry methanol at room temperature, erythromycin oxime (IV) is obtained in moderate yield.

The oxime is freed from unchanged I by recrystallization of the thiocyanate salt. After recrystallization from methanol the oxime base (IV), m.p. 157-59, exhibits a characteristic C=N absorption at 6.1 μ in the infra-red and has a pK_a 8.6 (66% DMF). The mass spectrum of IV shows a molecular ion at m/e = 748.

Catalytic reduction of IV, dissolved in aqueous solution containing two equivalents of δ-gluconolactone or in glacial acetic acid, with PtO_2 at 700 psi hydrogen pressure affords erythromycylamine (Va) in yields ranging from 60 to 95%. These are the only reaction conditions found satisfactory for this reduction, and these only when long reaction times (12-30 hrs.) and very large catalyst to substrate ratios (1:2) are employed.

The crude reaction product obtained from the catalytic reduction of IV usually contains one major component, erythromycylamine (Va), minor amounts of unchanged IV, and an isomeric substance (Vb). Pure Va is isolated by gradient pH extraction from pH 5.0-10.5 with methylene chloride and/or recrystallization of the 1,5-naphthalenedisulfonate salt. Homogeneous Va, m.p. 124-26 (ether), has pK_a's in 66% DMF of 8.7 and 9.9, molecular ion m/e = 734 in the mass spectrum, and has no residual absorption for C=N at 6.1 μ in the infra-red.

Treatment of Va in methanol with excess acetic anhydride affords a product which exhibits bands at 6.05 and 6.6 μ in the infra-red; these bands are characteristic for the CH_3CONH- function.

The co-product Vb from the reduction is isolated from the mother liquor after recrystallization of the 1,5-naphthalenedisulfonate salt. This substance, upon crystallization from ether, has m.p. 178-180, pK_a's of 8.4 and 9.7 (66% DMF), and shows the same molecular ion (m/e = 734) as does Va in the mass spectrum. Treatment of Vb with acetic anhydride as above likewise gives an amide-like product. Although the structure of Vb is not known with certainty, the above data suggests that it is the C-9 epimer (Vb) of erythromycylamine (Va).

The reports of Djokic, et al.,[1,2,3] cited above state that the reaction of erythromycin (I) with hydroxylamine hydrochloride in dry methanol in the presence of excess barium carbonate gave, after isolation and recrystallization from methanol, a substance, m.p. 184-89, which was called "erythromycin oxime." Reduction of this compound in methanol with sodium borohydride* was reported to give a substance which, after recrystallization from ether-pet. ether, had a melting point of 142-146 and a single pK_a of 8.4 (66% DMF)[3]. The latter "reduction" product was thought to be the corresponding "9-amino" analog.

Because of the apparent differences in physical properties of IV and Va with the supposedly corresponding species described by Djokic, et al.,[1,2], several attempts were made to duplicate the work reported by these authors. Using the barium carbonate oximation procedure we indeed obtained the higher melting substance (IVs), m.p. 188-191, which exhibited the properties ascribed to it, i.e., C=N absorption at 6.1 μ in the infra-red and pK_a 8.7 (66% DMF). However, the observation that a solution of IVs in 66% DMF has pH 6.7 as compared to pH 9.7 for the bases I and IV suggests that IVs is a salt**. The 60-MHz nmr spectra (DMSO-d) of IV and IVs are identical, except that the dimethylamino signal seen at 2.22 δ for IV has shifted downfield to 2.74 δ in the spectrum of IVs; this shift of -0.54 δ has been previously observed for protonated dimethylamino groups[5]. Treatment of IVs in aqueous methanol with dilute sodium hydroxide gave, after isolation and crystallization from methanol, the oxime IV, m.p. 157-59°. The latter was shown to be identical to a sample of IV prepared by our procedure by comparison of x-ray diffraction patterns.

The reaction of IVs with sodium borohydride and isolation of the "reduction" product following the procedure of the published reports[1,2]

* We can find no other examples in the literature of reduction of an oxime with sodium borohydride.

** Elemental microanalysis indicates that IVs contains one mole-equivalent of chlorine.

gave a material, m.p. 147-48 (ether-pet. ether), which was found to contain approximately 0.1% boron. This material, without further purification, was dissolved in dilute hydrochloric acid and the pH was adjusted to 2.5. The solution was maintained at room temperature for ten minutes. Reisolation and crystallization from methanol gave a compound (68%), m.p. 155-57, which by comparison of the respective powder x-ray diffraction patterns was shown to be identical with IV.

From the results of our work, we suggest that the "erythromycylamine" reported by Djokic, et al.,[1,2,3] is actually the oxime base (IV), liberated from the salt (IVs) by the basic, sodium borohydride reaction medium. If this is correct, we are unable to explain the further degradation studies and structural identifications reported[2] for the "amine".

We are continuing our investigation of erythromycylamine (Va), the epimeric base Vb, and derived compounds; reports describing the antimicrobial activities and further chemical and stereochemical studies of these aminomacrolides[6] will be forthcoming.

REFERENCES

1. S. Djokic and Z. Tamburasev, British Patent 1,100,504 (1968).
2. S. Djokic and Z. Tamburasev, Tetrahedron Letters, 1645 (1967).
3. Z. Tamburasev, G. Vazdar, and S. Djokic, Croatica Chemica Acta, 39, 273 (1967).
4. M. V. Sigal, Jr., P. F. Wiley, K. Gerzon, E. H. Flynn, U. C. Quarck, and O. Weaver, J. Amer. Chem. Soc., 78, 388 (1956).
5. J. Ma and E. Warnhoff, Can. J. Chem., 43, 1849 (1965).
6. For recent reports on the stereochemistry of 9-dihydroerythronolide species, see P. V. Demarco, Tetrahedron Letters, 383 (1969), and T. J. Perun and R. S. Egan, ibid., 387 (1969).