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# HALOGENATION OF $C_{16}$-MACROLIDE ANTIBIOTICS 17-HALOLEUCOMYCINS ($A_3$)

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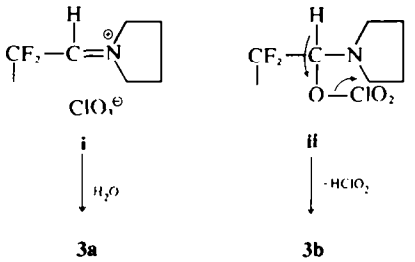
**Abstract**—The preparation of 17-bromo-, chloro- and fluoroleucomycin $A_3$ is described. Formation of a new cyclic ether was observed on pyrolysis of the 17-bromo system.

IN ASSOCIATION with earlier work with leucomycin $A_3$,[1] we had observed the facile formation of an enamine derivative **2** with pyrrolidine in benzene in the presence of molecular sieves at room temperature. On reexamination of this reaction it has been discovered that a very small amount of a by-product is formed simultaneously. The latter was isolated after acetylation by preparative TLC on silica gel and found in its PMR spectrum to lack the 3-acetoxyl function. In addition, the aldehyde proton exhibited a broad signal at $\delta$9.73 in contrast to that of leucomycin $A_3$ acetate itself. The mass spectrum confirmed this entity as leucomycin $A_3$ acetate *minus* AcOH and apparently identical with a product isolated by Omura *et al.*[2] by hot lithium hydroxide treatment of leucomycin $A_3$ followed by acetylation and formulated by these authors as the bicyclic system **4**. Formation of this product apears most logically rationalized as proceeding via initial $\beta$-elimination of the 3-acetoxyl group followed by Michael addition of $C_{17}$ to the resultant $\Delta^{\alpha,\beta}$-lactone. It is remarkable that this transformation proceeds under the extremely mild conditions of enamine formation albeit to a very minor extent.

More recently we have employed the enamine **2** as an appropriate intermediate for the selective introduction of halogen into the $C_{17}$-position. It appeared desirable to assess the possible biological potentiation of leucomycin $A_3$ as a consequence of fluorine substitution at this selectively accessible position.

Treatment of the enamine **2** in benzene or methylene chloride solution with perchloryl fluoride for 2–3 min at 25° yielded essentially two products which proved to be the difluoroaldehyde **3a** as its hydrate and the difluoropyrrolidine amide **3b**, respectively.[3] Even when fluorination was effected with less than one equivalent of perchloryl fluoride and under carefully controlled conditions of addition, no monofluoro product was evidenced. The mass spectra of these products, **3a** and **3b** after separation on silica gel were particularly informative. In the mass spectrum of leucomycin systems the parent ion *per se* is usually weak or more often undetectable. The major peak evidenced on the other hand is the parent ion *minus* the isovaleroyloxy group from the terminal mycarose glycoside, namely, the parent ion *minus* 101 mass units. Thus, **3a** exhibited a parent ion-101 peak at 762 and **3b** as its 9,2'-diacetate derivative correspondingly exhibited a parent ion-101 peak at 915. In neither instance was there evidence of peaks to be expected for mono-fluorinated systems. The FMR of the difluoroaldehyde, **3a**, in chloroform suggested an equilibrium between it and its hydrate as adjudged from the multiplicity of the fluorine signals centered around the major peak at 99 ppm. In addition **3a** formed a gem diacetate on acetylation with acetic anhydride in pyridine whereas thiosemicarbazide yielded a normal thiosemicarbazone derivative with the expected FMR spectrum. The structure of the pyrrolidine amide **3b** was evident from its amide band in the IR at 6.02$\mu$ as well as the absence of an aldehyde proton in the PMR. Formation of the amide **3b** could reasonably arise from the common immonium ion intermediate **i** derived from difluorination which hydrolyzes on the one hand to aldehyde **3a** and oxidizes in part via its carbinolamine equilibrium partner **ii** to yield amide **3b** in a manner comparable to the $\alpha$-elimination oxidations of chromic acid and hypohalites.[4]




In contrast to its behavior on fluorination, the enamine **2** reacted smoothly with N-chlorosuccinimide to yield, after hydrolysis, 17-chloroleucomycin $A_3$ **3c**. The latter exhibited a good parent ion-101 peak at 760 in its mass spectrum as well as a consistent NMR.

Bromination of leucomycin $A_3$ enamine **2** with N-bromosuccinimide proceeded in a parallel manner to the chlorination to provide 17-bromoleucomycin $A_3$ **3d**. The mass spectrum of the latter, however, unlike its chloro-counterpart did not give the anticipated parent ion-101 peak but instead exhibited a major peak conforming to parent ion-101 *minus* MeBr. This result suggested that cyclization with loss of MeBr was occurring during the ionization process to give the cyclic ether **5**. This conclusion was confirmed by submitting **3d** to brief pyrolysis at 180° whereby a new species was isolated possessing a mass spectrum identical with that of **3d** itself. The PMR of the latter, **5**, exhibited the absence of the C-4 OMe group as well as a doublet for the aldehyde proton.

The formation of the 17-monochloro- and monobromoleucomycin $A_3$ in contrast to difluorination by parallel procedure suggested a possible pathway to the 17-monofluoro system itself via a sequential halogenation followed by selective reduction. Leucomycin $A_3$ enamine

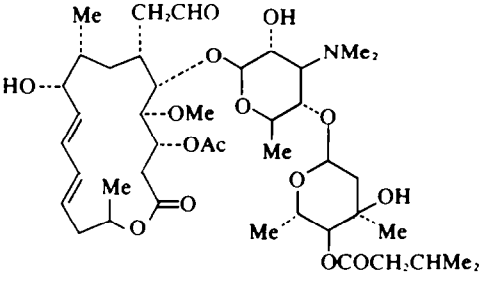
2 was, therefore, successively treated in benzene solution with N-bromosuccinimide (1 eq. 5°) followed by perchloryl fluoride (25°) and subsequent hydrolysis to yield a mixed bromofluoro species **3e**. The latter was directly reduced with triphenylphosphine[5] whereby only the Br atom was reductively removed. The product was purified on silica gel to give 17-fluoroleucomycin $A_3$ **3f** in 25–30% overall yield exhibiting the expected mass spectrum with parent ion-101 peak at 744.

The importance of the aldehyde function in 16-membered macrolides has been noted earlier by Omura and Nakagawa[6] and by Rakhit and Singh.[7] Loss of virtually all antimicrobial activity accompanies reduction to the corresponding carbinol. Results from our investigation indicate that fluorination $\alpha$- to the aldehyde reduces but does not abolish antibacterial activity. The fluoroaldehyde **3f** *in vitro* showed from one-half to one-twentieth the activity of the parent on fourteen leucomycin-sensitive strains of *Staph. aureus*, *Strep.* sp. and *Pasturella* Mult.

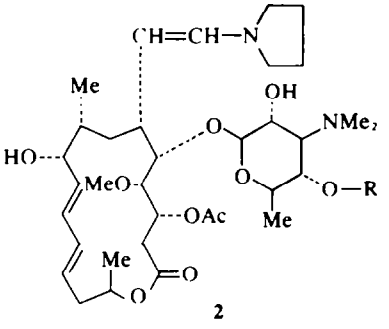
## EXPERIMENTAL

M.ps were taken on a microscope hot-stage apparatus and are uncorrected. UV spectra were determined in MeOH on a Cary Model 11 PMS spectrometer and IR spectra on a Perkin-Elmer Infracord instrument. PMR spectra were recorded on a Varian A-60 spectrometer using TMS as an internal standard and FMR spectra on a Jeolco C-60HL instrument using $C_6F_6$ on internal reference. Mass spectra were recorded on a LKB-9000 spectrometer at an ionization potential of 70 eV.

*Leucomycin $A_3$ enamine* **2**. A soln of 0.124 g (0.00015 mole) leucomycin $A_3$ and 0.064 ml (0.00075 mole) pyrrolidine in 0.3 ml dry benzene was stirred at 25° in the presence of 0.070 g molecular sieves (5A, 600 mesh). After 18 hr the mixture was filtered, the sieves washed with benzene and the filtrate evaporated to give 0.134 g of **2** (92%) as a foam. IR ($CHCl_3$) 6.07$\mu$. In addition PMR showed absence of the aldehydic hydrogen. Enamine **2** was used



**1**



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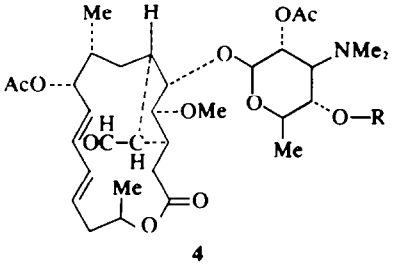

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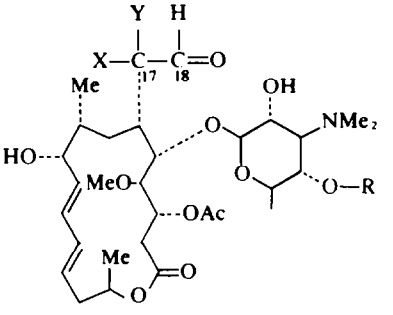
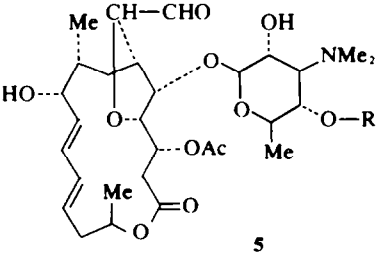


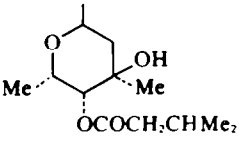
**3a**: X = Y = F (hydrate)

**3b**: X = Y = F; $C_{18}H$ = 

**3c**: X = H; Y = Cl
**3d**: X = H; Y = Br
**3e**: X = Br; Y = F
**3f**: X = H; Y = F



**5**

R = 

in subsequent experiments without further purification since it was found prone to facile hydrolysis on exposure to silica gel as well as to aqueous bicarbonate.

In one instance a soln of the enamine **2** (0.134 g) in benzene and ether was extracted twice with 10% $KHCO_3$, once with sat NaCl, dried ($Na_2SO_4$) and evaporated to give 0.117 g colorless foam, the IR and TLC of which were similar to those of leucomycin $A_1$. This material was acetylated (25°, 16 hr) with 0.5 ml pyridine and 0.5 ml $Ac_2O$. The crude product showed leucomycin diacetate and bicyclic aldehyde **4** as major and minor (mobile) components, respectively, in its TLC. Preparative TLC (silica gel, $C_6H_6$–acetone 75:25) provided 0.010 g (8%) of **4** which was identical with the material obtained by treatment of leucomycin $A_1$ with hot ethanolic LiOH–$H_2O$[2] followed by acetylation.

*17-Bromoleucomycin $A_1$* **3d**. N-Bromosuccinimide (0.0269 g, 0.00015 mole) was added to a stirred soln of **2** (obtained from 0.124 g, 0.00015 mole of leucomycin $A_1$) in 1.2 ml dry benzene at 5–7° (bath temp.) under $N_2$. The N-bromosuccinimide became yellow and amphorphous and dissolved in 10 min. After 30 min an aliquot showed a negative starch-iodide test. The mixture was diluted with ether, extracted twice with 10% $KHCO_3$, once with sat NaCl, dried ($Na_2SO_4$) and evaporated to give 0.149 g of colorless foam. Preparative TLC (silica gel, $C_6H_6$–acetone 60:40) provided 0.084 g (62%) of **3d** (single spot) as a colorless foam, the PMR ($CDCl_3$) of which indicated the presence of two diasteriomers in a ratio of 23:77 as adjudged by the presence of two doublets due to aldehydic proton at $\delta$9.57 (J = *ca.* 4 Hz) and $\delta$9.70 (J = *ca.* 3.5 Hz) $\lambda_{max}^{MeOH}$ 231 nm ($\epsilon$ = 26390); IR ($CHCl_3$) 2.73, 2.84, 3.68, 5.75, 5.80$\mu$; mass spec. $M^+$ not seen; $M^+$–$CH_3Br$, 811; $M^+$–$CH_3Br$–$C_5H_9O_2$, 710.3752. Calc. for $C_{36}H_{56}NO_{13}$, 710.3745. [Found: C, 55.62; H, 7.43; N, 1.42. Calc. for $C_{42}H_{66}O_{15}NBr$: C, 55.62; H, 7.56; N, 1.54%].

*Bromo ether* **5**. 17-Bromoleucomycin $A_1$ (0.068 g) was heated at 180° for 5 min in $N_2$ to yield **5** as essentially a single product by TLC, slightly more polar than **3d**. Preparative TLC (silica gel, $C_6H_6$–acetone 60:40) provided 0.030 g (50%) of pure **5**. PMR ($CDCl_3$) $\delta$9.59 (d, J = 2 Hz, CHO), 2.49 [s, $N(CH_3)_2$], 2.07 (s, OAc) and no signal due to OMe. $\lambda_{max}^{MeOH}$ 232 nm ($\epsilon$ = 20630); IR ($CHCl_3$) 2.84, 3.70, 5.75 and 5.80$\mu$; mass spec. $M^+$, 811; $M^+$–$C_5H_9O_2$, 710. [Found: C, 60.64; H, 7.92; N, 1.41. Calc. for $C_{41}H_{65}O_{14}N$: C, 60.64; H, 8.06; N, 1.72].

*17-Chloroleucomycin $A_1$* **3c**. N-Chlorosuccinimide (0.020 g, 0.00015 mole) was added to a stirred soln of **2** (obtained from 0.124 g, 0.00015 mole of leucomycin $A_1$) in 1 ml dry benzene at 25° in $N_2$. After 4 hr the mixture was diluted with ether, extracted with 10% $KHCO_3$ twice, sat NaCl once, dried ($Na_2SO_4$) and evaporated to give 0.130 g colorless foam. Preparative TLC (silica gel, $C_6H_6$–acetone 60:40) provided 0.085 g (66%) of **3c** (single spot) as a colorless foam. The PMR ($CDCl_3$) indicated the presence of two diasteriomers as adjudged by the presence of two doublets due to the aldehydic hydrogen at $\delta$9.62 (J = *ca.* 2 Hz) and 9.68 (J = *ca.* 2 Hz), the latter being the major signal. $\lambda_{max}^{MeOH}$ 231 nm ($\epsilon$ = 25960); mass spec. $M^+$ 861/863; $M^+$–$CH_3Cl$, 811; $M^+$–$CH_3Cl$–$C_5H_9O_2$, 710. [Found: C, 57.86; H, 7.88; N, 1.39. Calc. for $C_{42}H_{66}O_{14}NCl$: C, 58.48; H, 7.95; N, 1.62%].

*17-Fluoroleucomycin $A_1$* **3f**. N-bromosuccinimide (0.215 g; 0.0012 mole) was added to a stirred soln of **2** (obtained from 0.994 g, 0.0012 mole of leucomycin $A_1$) in 10 ml dry benzene at 5–7° under $N_2$. After 1 hr an additional 10 ml dry benzene was added and the soln exposed to a slow stream of $FClO_3$ for 3 min at 25° followed by $N_2$ for 5 min. The mixture was extracted with 10% $KHCO_3$ twice, sat NaCl once, dried ($Na_2SO_4$) and evaporated to give 1.080 g crude **3e**.

A soln of 1.080 g crude **3e** and 0.378 g (0.00144 mole) of $(C_6H_5)_3P$ in 5 ml dry ether was stirred at 25°. The mixture became heterogeneous in 0.5 hr due to separation of enol triphenylphosphonium bromide. After 24 hr the the mixture was diluted with 5 ml ether and stirred for 10 min in the presence of 12 ml of MeOH–$H_2O$ (9:3). The resulting homogeneous soln was evaporated at 25°, the residue dissolved in $C_6H_6$–ether, extracted with 10% $KHCO_3$ twice, sat NaCl once, dried ($Na_2SO_4$) and evaporated to give 1.415 g crude **3f**. Purification of the latter via dry column chromatography (silica gel 60 g, $CHCl_3$–acetone 70:30) provided 0.253 g (25%) *17-fluoroleucomycin $A_1$* **3f** as a colorless foam. PMR ($CDCl_3$) $\delta$9.77 (d, J = 6 Hz, –CHO), 2.56 [s, $-N(CH_3)_2$] and 2.13 (s, OAc); IR ($CHCl_3$) 2.85 and 2.78$\mu$. FMR ($CDCl_3$) resonance centered at 205 ppm. $\lambda_{max}^{MeOH}$ 231 nm ($\epsilon$ = 21740); mass spec. $M^+$ = 845; $M^+$–$C_5H_9O_2$ = 744.3980; Calc. for $C_{37}H_{59}O_{13}NF$ = 744.3969. [Found: C, 57.89; H, 7.93; N, 1.30. Calc. for $C_{42}H_{68}O_{15}NF\cdot H_2O$: C, 58.38; H, 8.16; N, 1.62%].

*17,17-Difluoroleucomycin $A_1$* **3a** *and difluoropyrrolidine amide* **3b**. A slow stream of perchloryl fluoride was passed through a stirred soln of **2** (prepared from 1.242 g, 0.0015 mole of leucomycin $A_1$) in 20 ml dry $CH_2Cl_2$ for 1.5 min at 25° followed by $N_2$ for 2 min. The mixture was extracted twice with 10% $KHCO_3$, once with $H_2O$, dried ($Na_2SO_4$) and evaporated to give 1.245 g yellow foam. This experiment was repeated twice, once with **2** derived from 1.242 g (0.0015 mole) leucomycin and once with **2** obtained from 1.863 g (0.00225 mole) leucomycin. The crude products from 3 runs were combined and weighed 4.180 g; TLC showed two major spots. Purification via dry column chromatography (silica gel 225 g, $C_6H_6$:acetone 60:40) provided 1.670 g (37%) of **3a** corresponding to the polar spot. The latter on long standing in ether at 0° yielded crystalline material m.p. 134–36°. $\lambda_{max}^{MeOH}$ 231 nm ($\epsilon$ = 26100); IR ($CHCl_3$) 2.84, 5.75 and 5.80$\mu$; PMR ($CDCl_3$) $\delta$9.53 (CHO, broad signal); FMR ($CDCl_3$) major peak at 99 ppm. Mass spec. $M^+$ = 863; $M^+$–$C_5H_9O_2$ = 762.3869, Calc. for $C_{37}H_{58}NO_{13}F_2$ = 762.3875. [Found: C, 58.74; H, 7.97; N, 1.47. Calc. for $C_{42}H_{67}O_{14}NF_2$: C, 58.38; H, 7.80; N, 1.62%].

Acetylation ($Ac_2O$, pyridine, 25°, 16 hr) of **3a** followed by preparative TLC (silica ge,, $C_6H_6$–acetone 75:25) provided *17,17-difluoroleucomycin*-9,2',18,18-*tetracetate*. IR($CHCl_3$) 2.84, 5.62, 5.72, 5.75, 5.79$\mu$. PMR ($CDCl_3$) $\delta$7.40 (m, C-18H), 3.45 (s, $OCH_3$), 2.38 [s, $N(CH_3)_2$] 2.19 (s, 1 OAc), 2.04 (s, 2 OAc), 1.97 (2 OAc). FMR ($CDCl_3$) $\Phi_{F_A}$ = 104 ppm, $\Phi_{F_B}$ = 111 ppm ($J_{F-F}$ = 273 Hz). Mass spec. $M^+$ not seen; $M^+$–$C_5H_9O_2$, 948. [Found: C, 57.01; H, 7.76; N, 1.19. Calc. for $C_{50}H_{77}O_2NF_2$: C, 57.18; H, 7.39; N, 1.33%].

Treatment of **3a** with 2 eqiv of thiosemicarbazide in refluxing EtOH for 24 hr provided the corresponding *thiosemicarbazone*. $\lambda_{max}^{MeOH}$ 231 nm ($\epsilon$ = 34580) and 277 ($\epsilon$ = 20520); PMR ($CDCl_3$–$CD_3OD$) $\delta$7.60 (m, C-18H). FMR ($CDCl_3$) $\Phi_{F_A}$ = 94 ppm. $\Phi_{F_B}$ = 99 ppm ($J_{F-F}$ = 279 Hz).

The material (0.940 g) corresponding to the mobile spot obtained during purification of **3a** was acetylated (3.5 ml pyridine, 3.5 ml $Ac_2O$, 25°, 16 hr) and found to be a mixture as indicated by TLC. Purification via a combination of dry column chromatography (silica gel 50 g, ether) and preparative TLC provided 0.150 g of **4** (identical with material obtained by treatment of leucomycin $A_1$ with LiOH–$H_2O$ in EtOH[2] followed by acetylation) and 0.25 g of **3b** as 9,2'-diacetate; $\lambda_{max}^{MeOH}$ 231 nm ($\epsilon$ = 29190). IR ($CHCl_3$) 2.83, 5.77 and 6.02$\mu$, PMR ($CDCl_3$) $\delta$3.47 (s, $OCH_3$), 2.41 [s, $N(CH_3)_2$], 2.17 (s, OAc), 2.03 (s, OAc), 1.98 (s, OAc); FMR ($CDCl_3$) $\Phi_{F_A}$ = 94 ppm, $\Phi_{F_B}$ = 109 ppm, ($J_{F-F}$ = 304 Hz). Mass spec. $M^+$ not seen, $M^+$–$C_5H_9O_2$ = 915. [Found: C, 58.83; H, 7.64; N, 2.42. Calc. for $C_{50}H_{78}O_{17}N_2F_2$: C, 59.04; H, 7.73; N, 2.75%].

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## REFERENCES

[1]N. N. Girotra and N. L. Wendler, *Tetrahedron Letters* 227 (1975).
[2]S. Omura, A. Nakagawa, R. Suzuki and T. Hata, *J. Antibiotics* **27**, 370 (1974).
[3]R. Verhé, N. DeKimpé, L. DeBuyck and N. Schamp, *Synthesis* **455** (1975) have recently described the formation of 2,2-dihalogenated aliphatic aldehydes by treating the corresponding Schiff bases derived from t-butyl amine with N-chlorosuccinimide.
[4]F. L. M. Pattison, R. L. Buchanan and F. H. Dean, *Can. J. Chem.* **43**, 1700 (1965) observed in the fluorination of aldehyde enol ethers the formation of fluoro esters as oxidation by-products.
[5]*cf* I. J. Borowitz, K. C. Kirby, Jr., P. E. Rusek and E. W. R. Casper, *J Org. Chem.* **36**, 88 (1971).
[6]S. Omura and A. Nakagawa, *J. Antibiotics* **28**, 401 (1975).
[7]S. Rakhit and K. Singh, *Ibid.* **27**, 221 (1974).