SYNTHESIS OF SOME DEGRADATION PRODUCTS OF ERYTHROMYCIN A

BY

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D E D I C A T E D

TO

My parents, brothers and sisters.

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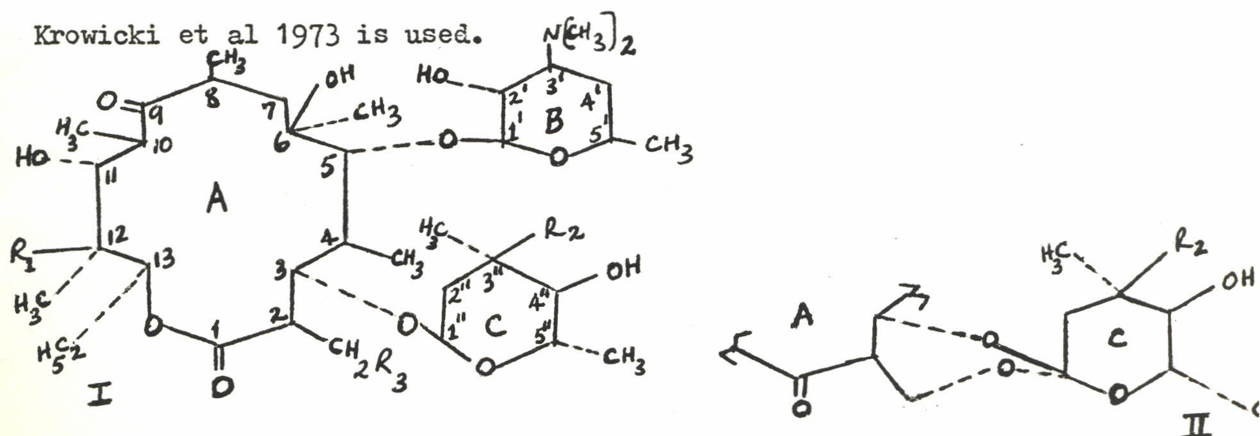
INTRODUCTION

Erthromycins are macrolide antibiotics produced by growth of certain strains of streptomyces erthreus. The general biosynthesis of erthromycin has been reported (Cane et al 1981). The naturally occuring erthromycin macrolide antibiotics consists of a multi-branched poly-functional 14 membered lactone ring substituted with an amino and a nitrogen free 6-deoxy sugar. Five erythromycins have been characterized. These are erythromycins A (Flynn et al 1954); B (Wiley et al 1957) C (Wiley et al 1957); D (Majer et al 1977); E (Martin et al 1975) and F (Martin et al 1982).

The stereochemical formular of erthromycin A has been presented in different ways. In the structure shown in (fig 1) the presentation of Krowicki et al 1973 is used.



	R_1	R_2	R_3	A	B	C
Erythromycin A	OH	OCH_3	H	Erythronolide A	Desosamine	Cladinose
Erythromycin B	H	OCH_3	H	Erythronolide B	Desosamine	Cladinose
Erythromycin C	OH	OH	H	Erythronolide A	Desosamine	Mycarose
Erythromycin D	H	OH	H	Erythronolide B	Desosamine	Mycarose
Erythromycin E	OH	OCH_3	O	Erythronolide A	Desosamine	Cladinose
Erythromycin F	OH	OCH_3	OH	Erythronolide A	Desosamine	Cladinose

The structure of Erythromycin E is as shown in II

Figure 1: Erythromycin structures

Erythromycin A, B, C and D are composed of two different 14 membered lactones; erythronolide A for erythromycin A and C and erythronolide B for erythromycins B and D; combined with a common basic sugar, D- desosamine and either of two neutral branch chain sugars L- cladinose (erythromycin A and B) or L- mycarose (Erythromycins C and D). Erythromycins E and F contain a hydroxyl and an ortho ester groupings respectively and represent a later stage of the erythromycin biosynthetic pathway.

Erythromycin A is the most potent antimicrobially of the erythromycins and is the main coponent in erythromycin samples used in medicine. Antimicrobial activity of erythromycin A, B, C and D have been reported by Majer et al (1977) and comparison of erythromycin A and E for different microorganisms by Martin et al (1975).

Antibacterial spectra, pharmacokinetics and Toxicity of Erythromycin

Erythromycin is active against Gram-positive bacteria especially pneumococci, hemolytic streptococci and corynbacterium diphtheriae and some gram-negative bacteria including Neisseria and Haemophillus species but not against the enterobacteria.

On absorption from the gastrointestinal tract it is rapidly distributed throughout the body fluids except for the cerebrospinal fluid. It is mainly excreted through the biliary route into faeces.

It was formerly used widely to treat infections caused by penicillin resistant staphylococci. However the newer penicillins have now taken its place. Due to rapid development of resistance its use is mainly restricted to the treatment of infections due to sensitive organisms in patients who are allergic to penicillins.

Some of its side effects include cholestatic jaindice and interference with the normal flora of the gastrointestinal tract leading to superinfections

Properties of Erythromycin

Erythromycins are sparingly soluble in water where solubility decreases with temperature increase. They are freely soluble in organic solvents or dilute hydrochloric acid. Crystals are readily obtained from aqueous acetone, aqueous alcohol or chloroform .

Erythromycin A is a base exhibiting a PKa of 8.6 (Koch; 1979) when titrated in 66% dimethyl formamide.

Acid stability

In acidic mediaerythromycin A rapidly loses its biological activity because of acid catalysed transformations. These transformations involve the formation of an internal enolether; 8,9 - anhydroerythromycin 6-9 hemiketal (Kurath et al 1971); by addition of the C-6 hydroxyl to the carbonyl at C-9 followed by dehydration with an introduction of a C-8-9 double bond.

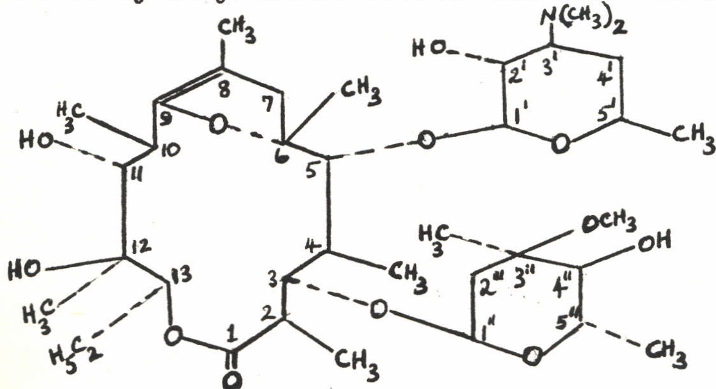


Fig 2: Erythromycin A enolether.

All the erythromycins;A, B, C, D, E and F undergo this degradation reaction to give the respective enolethers. Erythromycin A, C, E and F which have an hydroxyl group at C 12 degrade further by an irreversible addition of the C 12 hydroxyl group to the double bond at C8 - 9 to form anhydroerythromycin A (Kurath et al 1971).

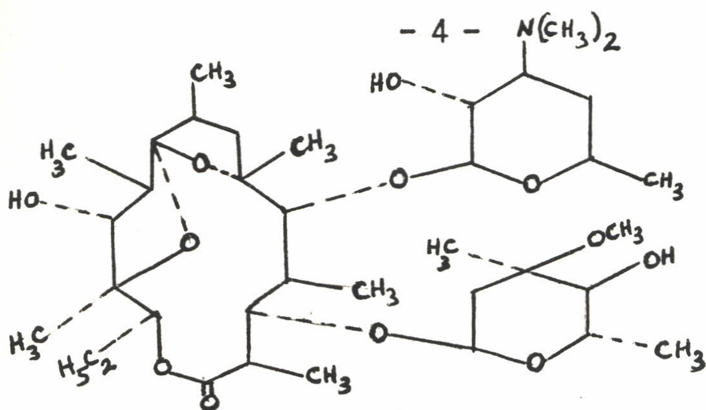
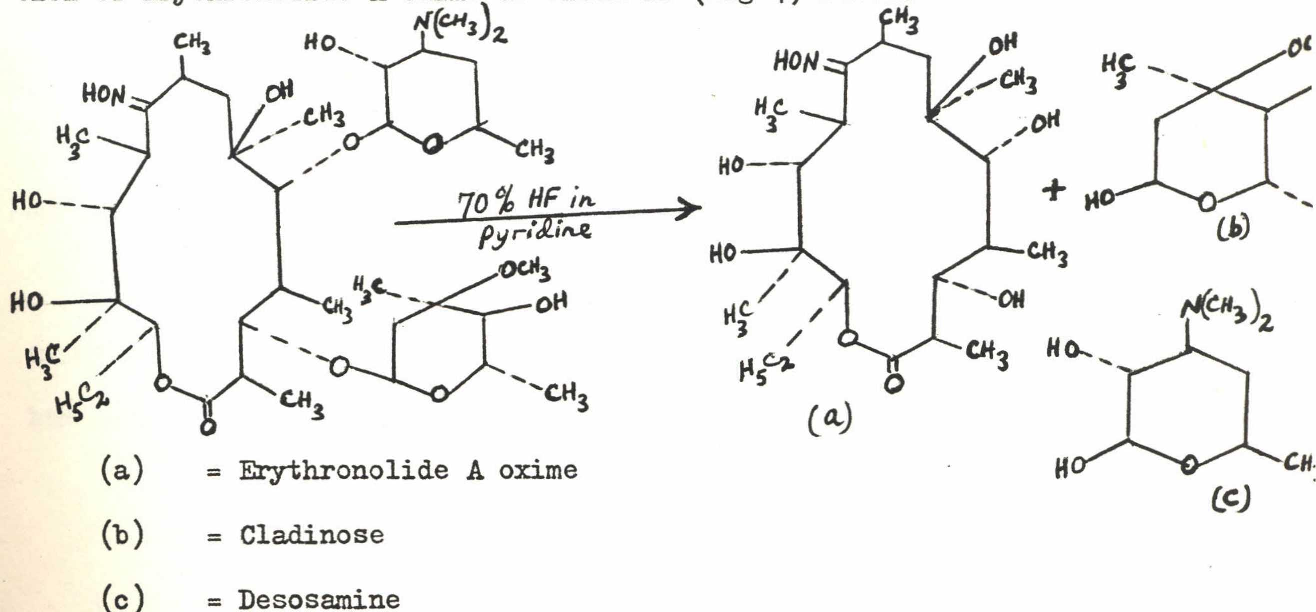


Fig 3: Structure of Anhydroerythromycin A.

Lemahieu et al (1974) have shown that these acid catalysed reactions can be prevented by blocking the carbonyl group at C-9 in their preparation of Erythronolide A Oxime as shown in (fig 4) below.



(a) = Erythronolide A oxime

(b) = Cladinose

(c) = Desosamine

Fig 4: Structure of Erythromycin A oxime, Erythronolide A Oxime, Cladinose and Desosamine.

Erythromycins lacking C-12 hydroxyl group are more stable than their counterparts since the irreversible conversion of enolether to spiroketal cannot take place.

On prolonged exposure to acid, the glycosidic linkage at the C-3 position is broken and the sugar cladinose (3-O-Methylmycarose) and erythralosamine are obtained.

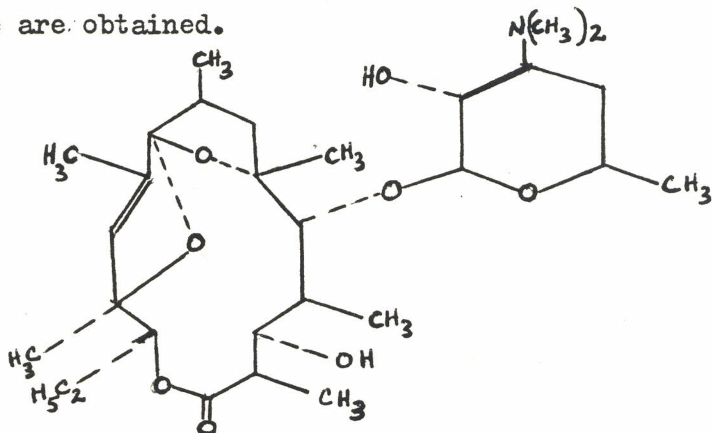


Fig 5: Erythralosamine

Enzymes in microorganisms inactive erythromycin forming erythralosamine and erythronolide (Flickinger 1975).

Demethylation of erythromycin A to form Des-N-Methyl erythromycin occurs in humans, rats, dogs and rabbits. The preparation of Des-N-Methylerythromycin has been reported by Flynn et al (1955)

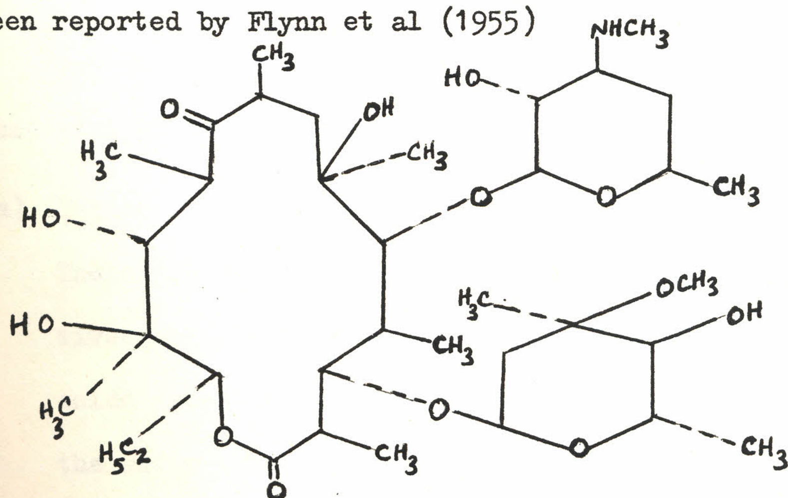


Fig 6: Structure of Des-N-Methylerythromycin A.

Other degradation products that have been isolated from mother liquor of fermentation broths include 5-O desosaminylerythronolide A and 15- norerythromycin C (Kibwage et al 1987).

The degradation products of eythromycin are biologically inactive or show limited activity as compared to erythromycin. The commercial preparations of erythromycins usually contain erythromycin A, B, C and ocassionally D. Erythromycin A is the major component and antimicrobially the most active. The extent of degradation of erythromycin samples in commercial preparations depend on the age, storage conditions and treatment.

Development of derivatives considered acid stable for oral use

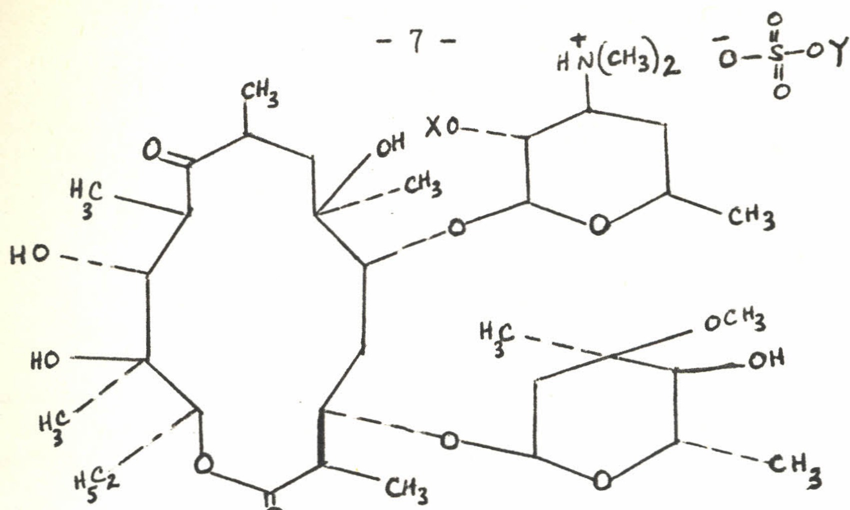
The most popular route of administration of preparations of erythromycin is the oral route. The intramuscular and intravenous routes of administration are suggested as alternatives. However these two routes of administration are not very popular due to the low solubility of the antibiotic or derivatives in aqueous solutions.

Due to the instability of erythromycin in acid, derivatives considered acid stable have been developed.

(a) Esters

These are by the far the most widely used derivatives. The derivatives are confined to the 2^1 esters of erythromycin Some of the acids used in the esterification process are also known to increase the water solubility of erthromycin and to modify its bitter taste.

A number of salts of propionylerythromycin and other erythromycin esters have been prepared and evaluated. Of the various salt types tested, the alkyl sulphate salts such as propionylerythromycin lauryl sulphate (PELS) (Stephens et al 1959); have been found to be very useful especially in the preparation of flavoured suspensions.



$X = CH_3CH_2\overset{O}{\overset{\|}{C}}-$

$Y = C_{12}H_{25}$

Fig 7: Structure of PELS

The water solubility of PELS is extremely low (about 0.024mg/ml) (Stephens et al 1959) and probably explains why the compund is substantially tasteless. PELS is a salt of a very strong acid (Lauryl sulphuric acid). The gastric acid is not strong enough to displace the acid radical of the salt and when mixed with gastric juice it remains undissolved and retains its potency even when periods of exposure are extended.

Erythromycin esters still in use in medicine include ethylerythromycin carbonate, propionylerythromycin and ethylerythromycin succinate. A summary of the gastric acid stability of erythromycin and esters given in the table below.

TABLE 1 Gastric juice stabilities

Compound	Exposure time in minutes	% activity retained at 3
1. Erythromycin (a)	5	3.5
2. Ethylcarbonylerythromycin (a)	5	5.7
3. Erythromycin Stearate (a)	5	2
4. Propionylerythromycin (b)	5	5.4
5. Propionylerythromycin Saccharinate (b)	15	10
6. Propionylerythromycin Lauryl sulphate(Erythromycin estolate) (b)	40	97.3

(a) PH 1.28

(b) PH 1.10

The potency of 2[1]esters of erythromycin has been investigated by Tardrew et al (1969) and found to be less than that of erythromycin. The apparent activity of 2[1] esters decreases in the order :-
Erythromycin; 2[1] - ethylsuccinylerythromycin, 2[1]- propionyl erythromycin 2[1]- benzoylerythromycin. This was observed to show inverse relationship with acid hydrolysis, which is expected since the 2[1] esters have to be hydrolysed to erythromycinto show activity.

(b) Use of enteric coating

Sylvester et al (1953) have demonstrated that a specially formulated acid-resistant coating could protect the drug from gastric secretions permitting the absorption of adequate amounts to produce serum concentrations of erythromycin well within the range necessary to inhibit most organisms sensitive to it. Use of enteric coating is most commonly used nowadays in the formulation of erythromycin for oral administration in adults.

AIM

Some of the deritives of erthromycin in the market are not enteric coated and could contain degradation products. These degradation products do not have antimicrobial activity thus making antimicrobial quantification hard.

One of the methods developed for quantification of the degradetion product is HPLC which necessiates the use of reference compounds

The objectives of this project are:-

(a) Synthesis and purification of some of the known degradation products that could be used as reference compounds.

(b) Comparative study of synthetic methods for one of these degradation products.

LIST OF REAGENTS AND SOLVENTS USED

REAGENT/SOLVENT	GRADE	SUPPLIER
Glacial Acetic acid	GPR	Kobian
Acetonitrile	Analar	Howse and Mc George
Aqueous Ammonia	GPR	Howse and Mc George
Anisaldehyde	GPR	Kobian
Chloroform	GPR	Kobian
Dichloromethane	GPR	Kobian
Diethylether	GPR	May and Baker
Erythromycin A	Commercial	Roussel Uclaf (France)
Ethanol (absolute)	Analar	Kobian
Ethylacetate	GPR	May and Baker
n - Hexane	Analar	Kobian
Hydrochloric acid	Analar	Howse and Mc George
Methanol	Analar	Kobian
Phosphorus pentoxide	Laboratory Reagent	Howse and Mc George
Potassium bromide	Laboratory reagent	May and Baker
Pyridine	GPR	Howse and Mc George
Sodium Bicarbonate	Laboratory reagent	Kobian
Sodium hydroxide	Laboratory reagent	E. T. Monks
Anhydrous Sodium Sulphate	Analar	E. T. Monks
Sulphuric acid	Laboratory Reagent	Howse and Mc George

GPR = General Purpose Reagent

Analar = Analytical

1. SYNTHESIS OF ACID DEGRADATION PRODUCTS OF ERTHROMYCIN A

The methods of synthesis of some of the known acid degradation product of erythromycin A have been modified and reported. (Kibwage I. O 1982) and are used here.

The starting material is commercial erythromycin A supplied by Roussel Uclaf (France).

Purification of erythromycin A

About 50g of erythromycin A was dissolved in ten parts by volume dichloromethane and the solution kept at -15°C for 24 hours. The crystals formed were recovered by filtration through a sintered glass filter and air dried. The crystallized erythromycin A melted at 140-143°C (all melting points were determined by a GallenKamp apparatus and are uncorrected). Literature melting point of erythromycin A is 135-140°C (Flynn et al 1954)

Preparation of Anhydroerythromycin A

4g of purified erythromycin A was suspended in 100ml of distilled water and the PH adjusted to 2.5 with concentrated hydrochloric acid at which point all the erythromycin dissolved. The solution was left at room temperature for about 30 minutes. 50ml of dichloromethane was added and the acidic media neutralized first by 0.5N sodium hydroxide solution until PH of about 6.5, followed by sodium bicarbonate to about PH 8.5 . The diphasic mixture was transfered into a separatory funnel and the organic phase run off. The aqueous phase was extracted two more times with the same amount of dichloromethane. The organic phases were combined, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure.

The residue was dissolved in 20ml dichloromethane and 20ml n-hexane

added. Crystals, formed overnight at -15°C, were recovered by filtration and recrystallized once more. In each case the crystals were washed with a cold mixture of dichloromethane - n - hexane(1:1). After air drying the crystals the yield and melting point were obtained.

Yield 2.0g (51.3%)

Melting point 112-116°C

Literature melting point 110-120°C (Wiley et al, 1957)

Preparation of Erythralosamine

4g of purified erythromycin A was dissolved in 200ml of 0.75N hydrochloric acid. The solution was let at room temperature for 20 hours. It was neutralized first with 0.5N sodium hydroxide solution then with solid sodium bicarbonate. The erythralosamine formed was extracted three times with 50ml of dichloromethane. The organic phases were combined, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue was dissolved in n-hexane and put at -15°C for 24 hours. Crystals were recovered by filtration through sintered glass filter after which they were air dried.

Yield 2.0g (57.6%)

Melting point 197-200°C

Literature melting point 200-203°C (Flynn et al. 1954)

Preparation of erthromycin A enolether

About 20g of erythromycin A was weighed into a beaker and dissolved in 15ml Glacial acetic acid. The solution was left at room temperature for 2 hours.

About 100ml of distilled water was added and mixture transferred to a 500ml separatory funnel containing 50ml of dichloromethane. To neutralize the acid, excess sodium bicarbonate (25g) was added in small quantities with shaking. The organic layer was run off and the aqueous phase extracted two more times each with 50ml of dichloromethane. The organic phases were combined dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure.

The enolether residue was crystallized twice from a minimal amount of warm absolute ethanol at -15°C for 24 hours. Crystals were recovered by filtration and air dried.

Yield 0.73g (37.4%)

Melting point 135 - 138°C

Literature melting point 138 - 140°C (Kurath et al 1971)

<u>Comparison of synthetic methods for Erythromycin A enolether</u>

It is a common principle that change of reaction conditions and solvents can influence the rate of production of a given product. Where more than one product is produced the reaction conditions tend to influence the preferential production of one over the other.

Erythromycin A enolether was synthesized through a new method, described below.

10.0g of purified erythromycin A was dissolved in 150ml of a 3:1 mixture of pyridine and Glacial acetic acid and heated for 24 hours at 70°C. After cooling the mixture was diluted with 500ml of saturated sodium bicarbonate solution and 50ml of dichloromethane added.

The aqueous phase was extracted two more times with 50ml of dichloromethane. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to dryness. Traces of pyridine were removed by co-distillation with n-hexane. The weight of the dry foam was 6.0g Being a foam the product could not be easily purified by crystallization. It was necessary to run column chromatography on the product but to establish which eluates contained what product, 0.5g of foam was column chromatographed. The mobile phase was dichloromethane-methanol-25% Ammonia (100:8:0.5) V: V: V. The stationary phase was analytical grade Silica gel for column chromatography.

The volume of each eluates collected was approximately 16.0ml. The different eluates were spotted on TLC to establish the product (s) in them. The results are as shown below.

<u>Results and discussions for the comparative synthetic method of erthromycin A enolether</u>

<u>Table 2</u>

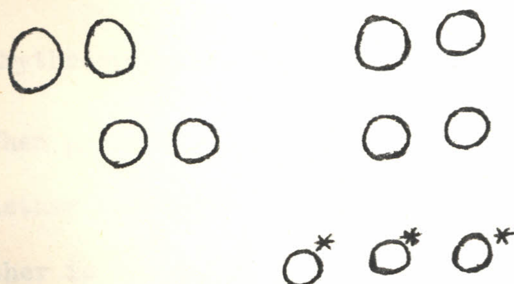
<u>RF Values of the different fractions</u>

Fraction	Rf value	Colour of spot
12	0.64	Grey - green
16 - 34	0.56	Grey - green
35 onwards	0.51	Grey - green

Fig 8.

TLC CHROMATOGRAMS OF COLUMN CHROMATOGRAPHY ELUATES

MOBILE PHASE: Ethylacetate - Methanol - 25% Ammonia (85:10:5) V:V:V ... SOLVEN



12 13-15 16-34 35-40 A B

12 - Eluate collected in tube labelled '12'

13 - 15 Eluates collected in tubes labelled 13 - 15

16 - 34 Eluates collected in tubes labelled 16 - 34

35 onwards - Eluates collected in tubes labelled from 35 onwards

A - Reaction mixture or crude product

B - " " " " "

* - Major product of the reaction.

The synthesized compound gave three spots on TLC. The compound eluting between 12-15 was erythromycin A enolether, Rf 0.64. The fractions eluting from 35 onwards correspond to the desired compound which has been identified as pseudoerythromycin A enolether (Kibwage I.O; personal communication).

Pseudoerythromycin A enolether and erythromycin A enolether are interchangeable into one another and usually exist in equilibrium in pyridine/acetic acid mixture.

Erythromycin A enolether $\rightleftharpoons$ pseudoerythromycin A enolether

When pyridine is the major solvent the formation of pseudoerythromycin A enolether is favoured; whereas in aqueous solution erythromycin A enolether is the major product. Further work on this synthesized compound was not done due to lack of time.

II CONTROL OF PURITY OF SYNTHESIZED PRODUCTS BY THIN LAYER CHROMATOGRAPHY

The separation of compounds in Thin layer chromatography (TLC) is based on the principle of either adsorption or partition.

TLC has achieved phenomenal success since its discovery in investigating column methods, its rapidity, excellent resolving power and its application to analytical problems and preparative work for which thicker layers can be used . (Beckett and Stenlake, 1976).

Several thin layer chromatograhy systems were recently reported (Kibwage et al, 1983) for the separation and identification of erythromycins and their degradation products. Some of the mobile phase systems that have been used widely include:-

(a) Dichloromethane - 95% aqueous methanol - Ammonia hydroxide (90:10:1) V:V:V

(b) Chloroform - methanol - 17% Ammonia (2:1:1) V:V:V:

(c) Chloroform - ethanol - 35% Ammonia (85:15:1) V:V:V

(d) Ethylacetate - Methanol - 25% Ammonia (85:10:5) V:V:V

(e) Dichloromethane - methanol - 25% Ammonia (90:9:1.5) V:V:V

(f) Diethylether - methanol - 25% Ammonia (90:9:2) V:V:V

(g) Diisopropylether - methanol - 25% Ammonia (75:35:2) V:V:V

Mobile phases (d) - (g) are amongst those that have been recently reporte

Mobile phase (d) has been shown to give good separation of all the components existing in commercial samples except erthralosamine and anhyroerthromycin where partial separation occurs. Enolethers of erthromycin A and B are also not well separated. Mobile phase (g) has been shown to give excellent separation between erthromycins A, B, C, D, anhydroerythromycin A and erythromycin A enolether.

However where erythromycins A and B enolethers are present there is partial separation between them. This mobile phase gives better separation at low temperatures of about 7 °C. With mobile phase (f) the separation of erythromycin A and erythromycin B is better but anhydro-erythromycin A is not separated from erythromacin A.

Experimental

Chromatographic material

Silica gel 60 HF_{254} for TLC (E. Merck, Art 7739)

Preparation of plates

About 35g of the adsorbent (Silica gel 60 HF_{254} for TLC, E. Merck, Art 7739) was weighed and about 70ml of distilled water added. The mixture was shaken until a slurry of good consistency was obtained and transfered quickly to a Dasaga spreader (C. Desaga GMBH).

The slurry was spread over the plates to give a thickness of 0.25mm. The plates were activated at 110°C for 1 hour. They were then cooled and either used immediately or stored in a desicator over silica gel.

Mobile phase

Ethylacetate- methanol - 25% Ammonia (85:10:5) V:V:V

Spray reagent

Anisaldehyde - concentrated sulphuric acid - Ethanol (1:1:9) V:V:V.

Method

About 5 ml solutions containing 10mg of product in 1 ml dichloromethane were applied to the Silica gel plates by means of capillary tubes.

The plates were developed through a pathlength of 15cm in chromatographic tanks lined with filter paper and saturated for the least one hour prior to developing the plates.

After development plates were heated in an oven at 110°C for 2 minutes cooled sprayed and returned to the oven for one minute.

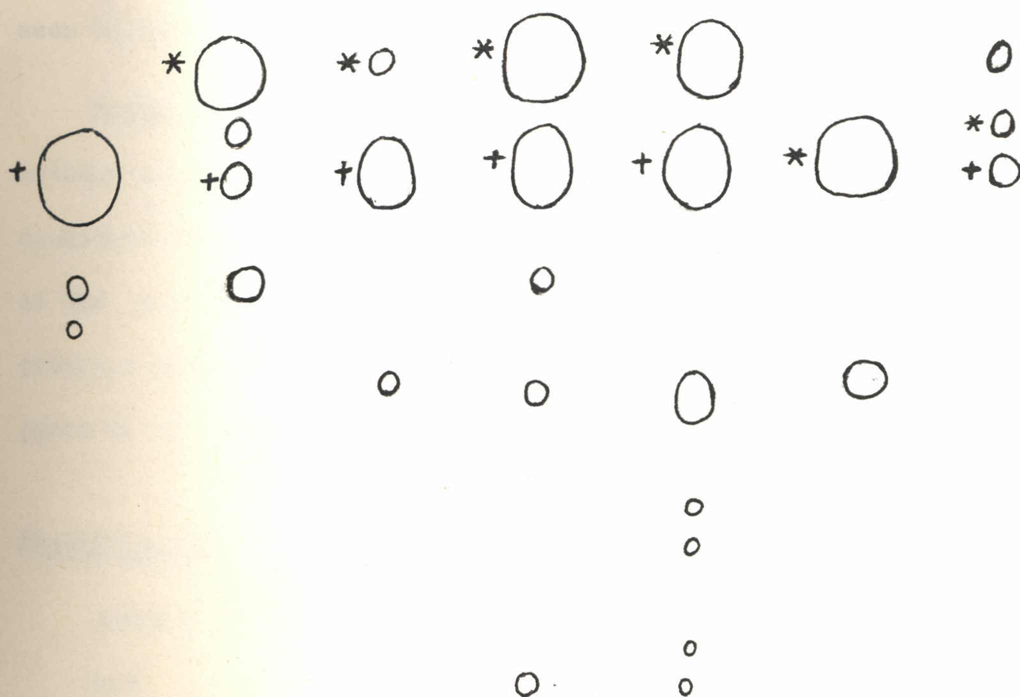
RESULTS AND DISCUSSIONS

The synthesized product was considered pure when only one spot was detected on TLC for that particular product. The reaction was deemed complete when no spot corresponding to erythromycin A, the starting material could be detected from the spotted crystallization Mother liquor sam It was also possible to assess whether the intended product had fully crystallized out. The chromatograms of these synthesized compounds are shown below.

Fig 9. TLC CHROMATOGRAMS OF SYNTHESIZED COMPOUNDS

MOBILE PHASE: Ethylacetate - methanol - 25% Ammonia (85:10:5) V:V:V

SOLVENT FRON



1 2 3 4 5 6 7

1. Erythromycin A
2. Erythralosamine
3. Mother liquour from which erythralosamine crystallized
4. Erythromycin A enolether
5. Mother liquor from which erythromycin A enolether crystallized
6. Anhydroerthromycin A
7. Mother liquor from which anhydroerythromycin A crystallized

* - Major spot of the desired product

\+ - Erythromycin A.

On TLC erythromycin A enolether gave 5 spots, Erythralosamine gave 4 spots whereas anhydroerythromycin A gave two spots. When the mobile phase chloroform - methanol-25% Ammonia (90:10:1) V:V:V was used it showed less separative power on the products (gave fewer spots on TLC) compared to the mobile phase Ethylacetate-methanol-25%Ammonia (85:10:5:) V:V:V. In all other subsequent work the latter was used. The reactions for erythromycin A enolether and erythralosamine were not complete because spots of the same colour and Rf value to that of erythromycin could be seen in the spotted mother liquor samples for these two.

Repeat syntheses for these two was done in which case the dichloromethane was first distilled. Phosphorous pentoxide was added to the dichloromethane before the distillation was done to help in removing some of the organic impurities. Same synthetic procedures were used as in previous synthesis. The yields and melting points were obtained and compared to the previous ones for two .

Erythralosamine

Yield = 2.37g

M.P = 199-200°C

Erythromycin A enolether

Yield = 0.7g

M.p = 140°C

The melting points are uncorrected.

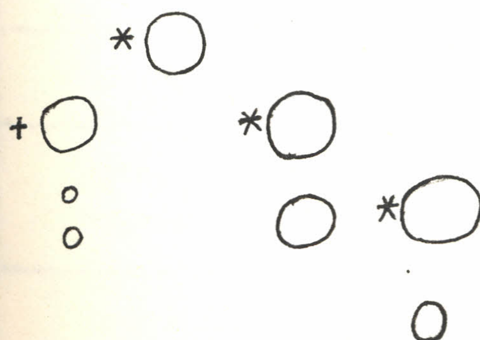
Both showed higher purity (mp range) compared to the previous samples obtained. The yield for erythralosamine was higher (2.37g) compared to the previously obtained one (2.0g).

For erythromycin A enolether the yield was slightly lower; 0,70g compared to 0.73g.

Fig 10

TLC CHROMATOGRAMS OF ACID SYNTHESIZED DEGRADATION PRODUCTS OF ERYTHROMYCIN A

MOBILE PHASE: Ethylacetate-methanol-25% Ammonia (85:10:5) V:V:V SOLVENT FRON



1 2 3 4

1. Erythromycin A
2. Erythromycin A enolether
3. Erythralosamine
4. Anhydroerythromycin A

* - Major spot of the derived product

\+ - Erythromycin A spot

ed compounds obtained from TLC on Silica gel
ethanol-25% Ammonia (85:10:5) V:V:V

Rf of spot	Colour of spot
+ 0.64 0.59 0.55	Grey-green Faint violet Faint violet
* 0.71	Grey-green
* 0.64 0.56	Orange Violet
* 0.57 0.47	Grey-green faint violet

the derived product

for the erythralosamine, one was orange the
The orange spot (Rf 0.64) is due to
one giving Rf of 0.56 is due to cladinose which
esulting from acid degradation of erythromycin

COMPOUNDS

red spectrum may be
means of identification.
ectra agree in all
sity of the bands. If
ained by examination of
must be carried out. The
tional tests.

the same solvent and

solvent and measurement
lvent as blank. This is
er the same conditions.

spectra of the synthesized Com-
ature. The fine differences
the sample used together
us used.

model 727 B series.

rtar and triturated with 200mg
) until a fine powder was
a Carver press, Model C,
inutes under vacuum.

RESULTS AND DISCUSSIONS

The IR spectra of the compounds are shown, figs 11-14.

Basically the absorption in the region of over 1450 cm^{-1} (i.e 1450 - 600 cm^{-1}) for all the compounds synthesized and erythromycin A is the same. The differences observed in this region are minor.

TABLE 4

Wavelength of absorption and the corresponding functional groups for erythromycin A

Wavelength of absorption (cm^{-1})	Functional group
3400-3500	OH groups
2700-3000	$-CH_2-$ and $-CH_3$ Groups
1720	Lactone group
1700	Ketone group

The peak at 1700cm^{-1} in Erythromycin A is not observed in erythromycin A enolether, Anhydroerythromycin A and erythralosamine.

Below 1450 cm^{-1} discrete differences are observed. This region serves to indicate differences between compounds but cannot be used for structural interpretatation due to complexity of erythromycin and its degradation products.

Erythromycin A enolether

The absorption band at about 1410 cm^{-1} is pronounced than in erythromycin A. The absorption band at 1340 cm^{-1} in erythromycin A is shifted to 1330 cm^{-1} in enolether.

Enolether has a strong absorption between 1200 - 1300 cm^{-1} compared to erythromycin. At about 1580 cm^{-1} the absorption is stronger for erythromycin than for enolether.

At 1730 cm^{-1} enolether shows a stronger absorption than erythromycin. At 1650-1630 cm^{-1} enolether shows absorption whereas erythromycin does not. This arises due to the $C_8 - C_9$ double bond in enolether (Fig 2)

Anhydroerythromycin A

At 1730 cm^{-1} there is a strong band compared to erythromycin A. Erythromycin shows a single strong peak at 1500 cm^{-1} compared with anhydroerythromycin which has split weak bands (3) around this wavelength.

There are slight differences between 1500 cm^{-1} and 1200 cm^{-1} for both anhydroerythromycin and Erythromycin.

At 905 cm^{-1} a stronger band is observed in anhydroerythromycin A compared to erythromycin and enolether. This band is common of compounds with the spiroketal group (fig 3 and fig 5).

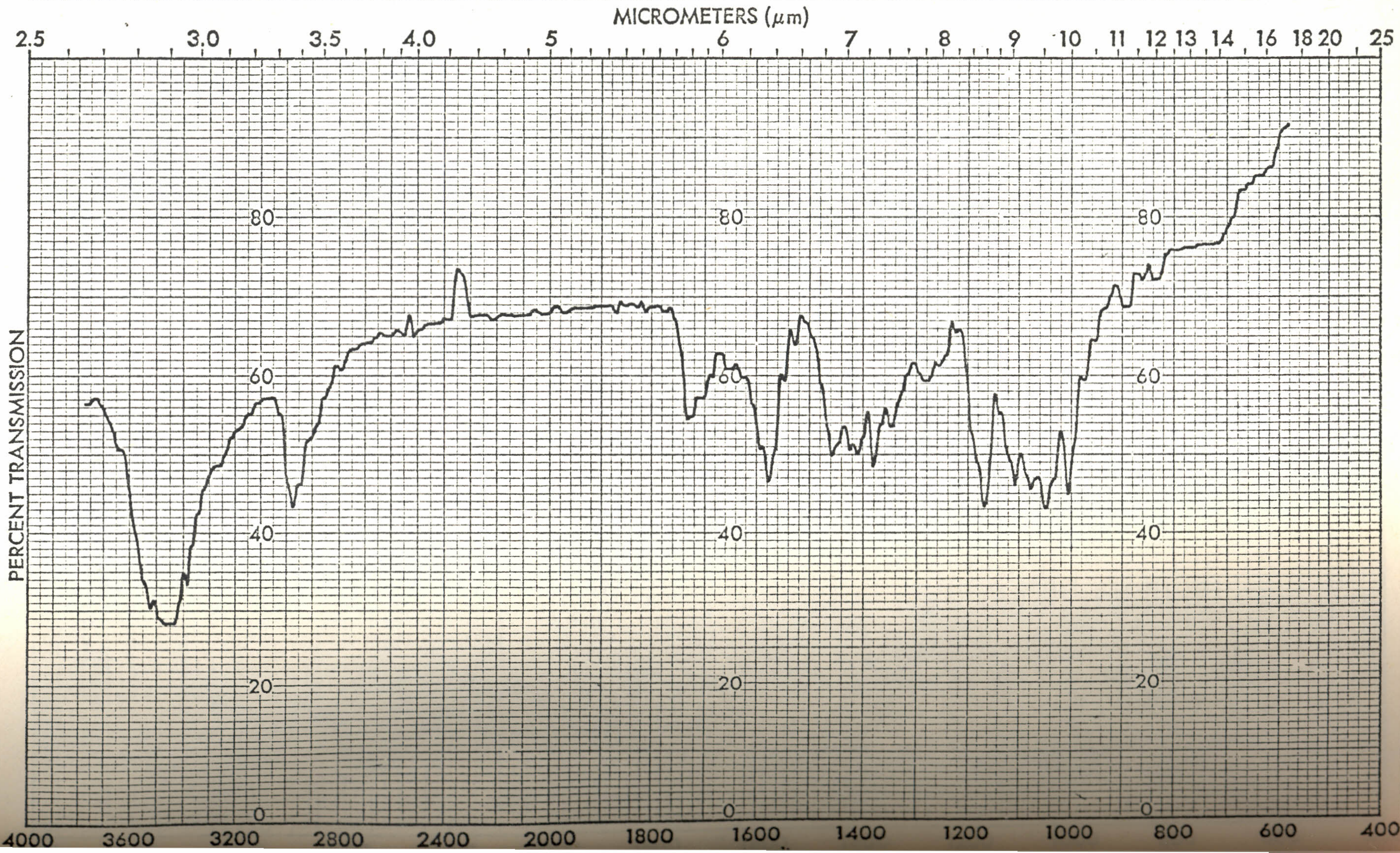
Erythralosamine

At 905 cm^{-1} there is a strong peak which is also present in anhydroerythromycin. Between 1700 cm^{-1} and 1600 cm^{-1} strong absorption bands are observed. These bands at same region, are weaker for both erythromycin A and anhydroerythromycin.

Between 1500 cm^{-1} - 1240 cm^{-1} erythralosamine shows several strong absorption bands compared to erythromycin.

The IR spectra indicate that the synthesized compounds are chemically differenct from each other. The differences in intensity of absorption at same regions may not necessarily indicate different functional groups. This can arise due to the differences in the relative instrument response in various regions of the spectrum.

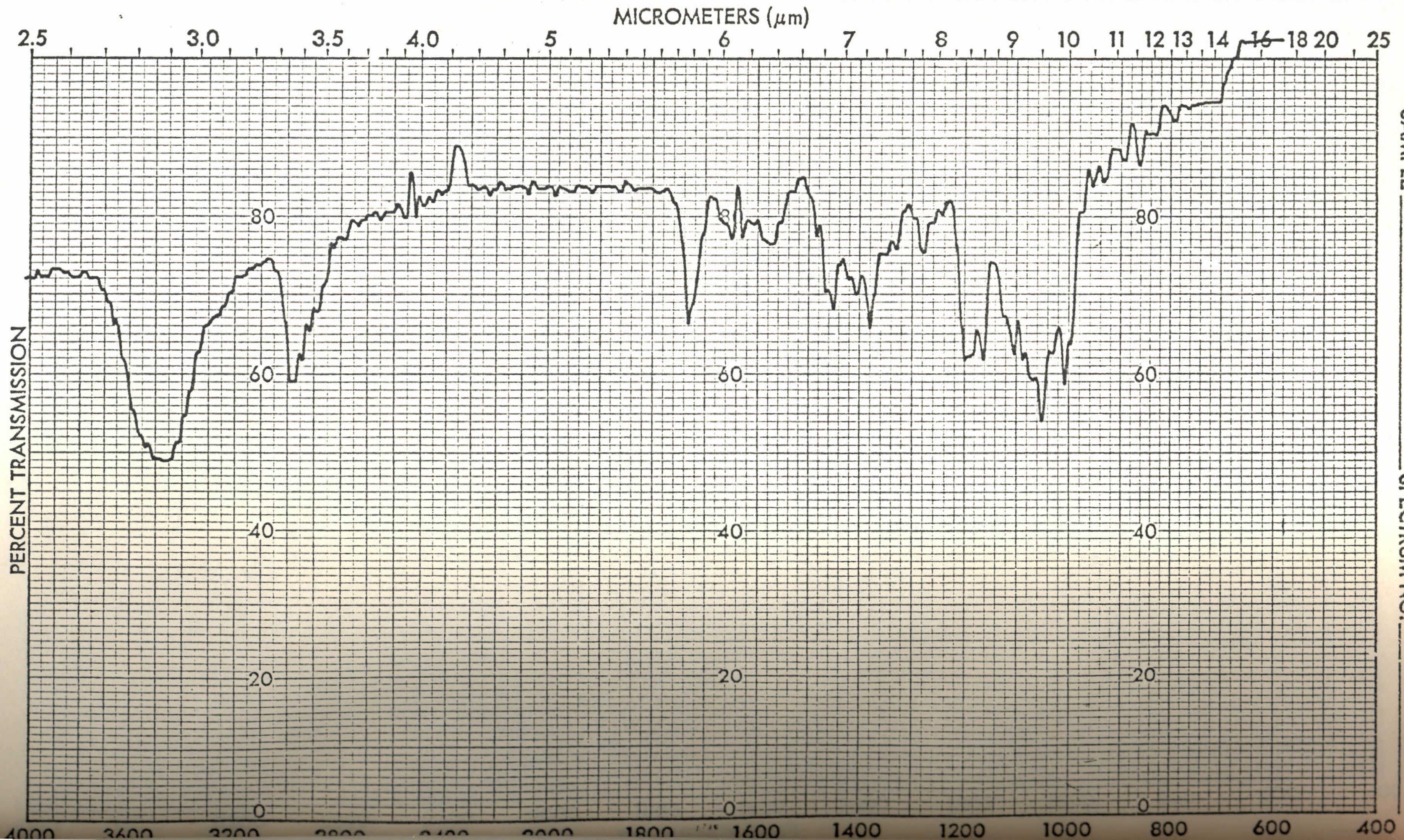
From both TLC and IR spectra evaluation the synthesized compounds are confirmed to be the same as those given in literature.



NO. 007-1493
PERKIN-ELMER
CONCENTRATION
THICKNESS
PHASE
REMARKS
SCAN MODE
ACCY.
HI ENERGY
RESOLUTION
SURVEY
CAL.
OPERATOR KAMAN J.N
DATE 26.2.87
SPECTRUM NO. Fig. 11.
SAMPLE ERYTHROMYCIN A
ORIGIN
MICROMETERS (µm)
2.5
3.0
3.5
4.0
5
6
7
8
9
10
11
12
13
14
16
18
20
25
PERCENT TRANSMISSION
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
SAMPLE
SPECTRUM NO.

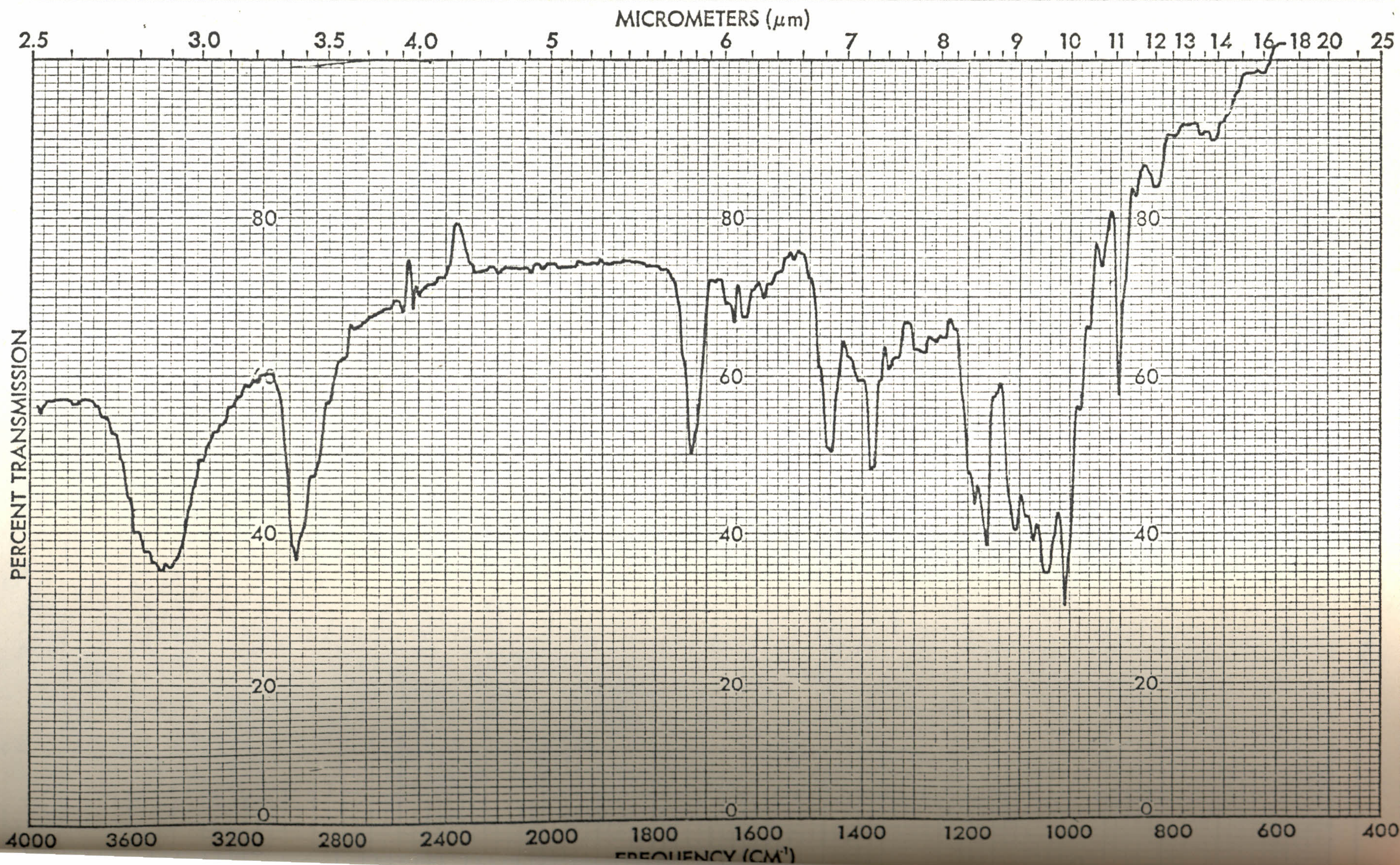
NO. 007-1493

PERKIN-ELMER

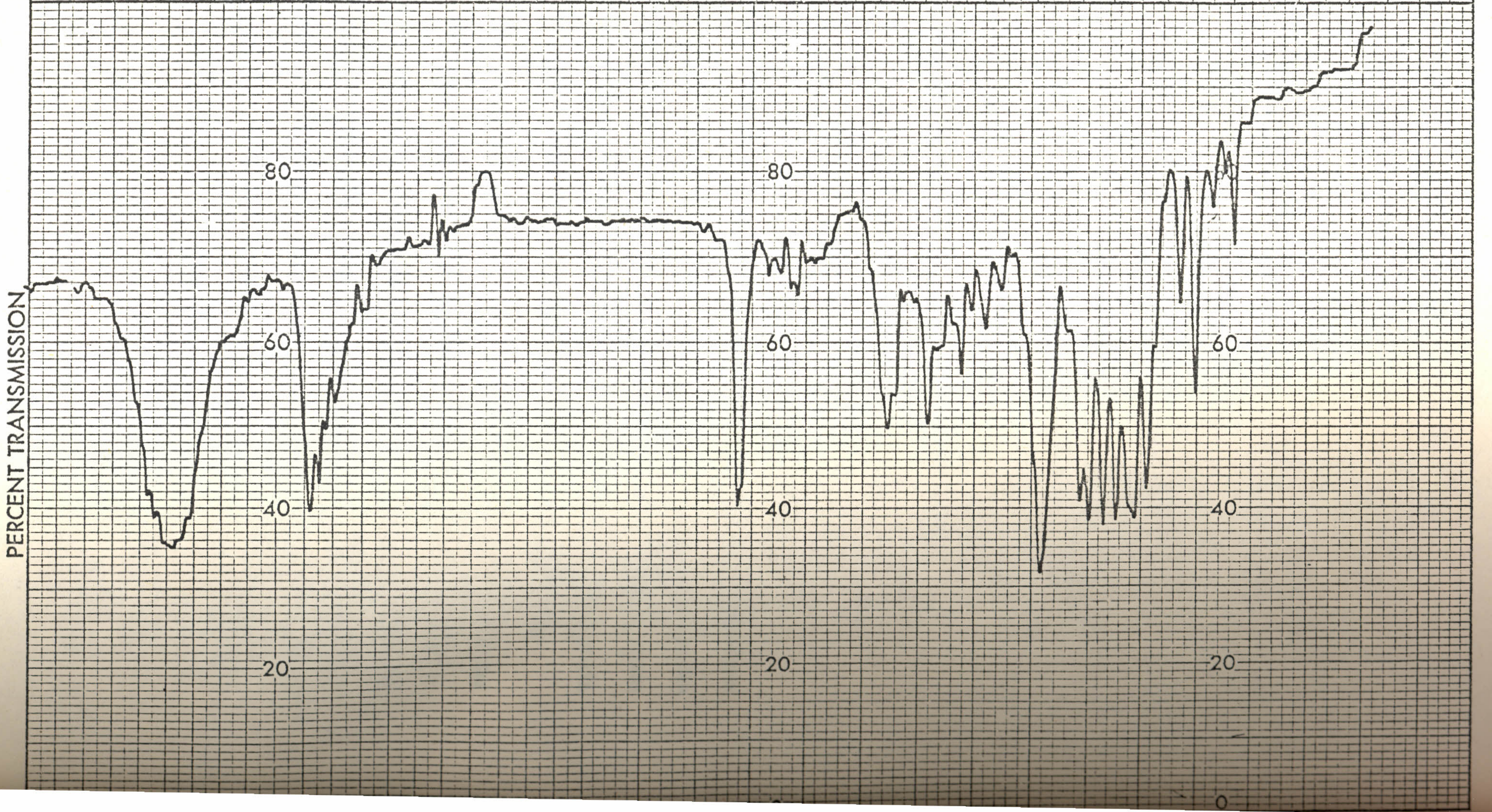


NO. 007-1475

CONCENTRATION	SCAN MODE ACCY. ☐ SURVEY ☐	SPECTRUM NO. Fig 13.
THICKNESS	HI ENERGY ☐ CAL. ☐	SAMPLE ANHYDROERYTHROMYCIN A
PHASE	RESOLUTION ☐	
REMARKS	OPERATOR KAMAU J.N DATE 26.2.87	ORIGIN



CONCENTRATION	SCAN MODE	ACCY. ☐ SURVEY ☐	SPECTRUM NO. Fig. 14
THICKNESS		HI ENERGY ☐ CAL. ☐	SAMPLE ERYTHRALOSAMINE
PHASE		RESOLUTION ☐	
REMARKS	OPERATOR KAMAU T.N	DATE 26.2.87.	ORIGIN



SUMMARY

During the course of this work it has been observed that synthesis of acid degradation products of erythromycin A is accompanied by impurities which could be intermediates or isomeric and epimeric forms of the synthesized compounds. The impurities could also arise due to the low purity of the starting material, solvents and reagents. Only one product, erythromycin A enolether (fig 10) is obtained pure.

The mobile phase dichloromethane - methanol - 25% Ammonia (85:10:5) V:V:V used in column chromatography is established to separate well the three compounds obtained during the comparative study of methods for erythromycin A enolether. This reaction yields pseudoerythromycin A enolether with erythromycin A enolether as one of the impurities.

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