A NEW CATIONIC ANTIMICROBIAL AGENT, *N*-DODECYL-4-AMINOQUINALDINIUM ACETATE (LAUROLINIUM ACETATE)

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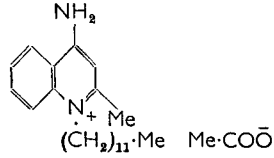
The properties of a new cationic antimicrobial agent, *N*-dodecyl-4-aminoquinaldinium acetate (laurolinium acetate, Laurodin) are described. It has bacteriostatic and bactericidal activity against Gram-positive and Gram-negative bacteria, fungi and some protozoa. Its activity is antagonised by anionic substances but some is retained in the presence of organic matter. The compound is well suited for topical use but is too toxic for parenteral use.

THE antimicrobial activity of the polymethylene bis-4-aminoquinaldinium series of compounds has been studied by Babbs, Collier, Austin, Potter and Taylor (1956), Collier, Cox, Huskinson and Robinson (1959), and Cox and D'Arcy (1961). The allied *N*-alkyl derivatives of 4-aminoquinoline and 4-aminoquinaldine have antibacterial activity (Cox and D'Arcy, 1959; Caldwell, Cox, D'Arcy and Rowe, 1961). In particular, the *N*-dodecyl acetate member of the 4-aminoquinaldine series (laurolinium acetate, Laurodin†) was shown to have bacteriostatic and bactericidal activity. This has been further studied and the results are now described.

EXPERIMENTAL

Materials and Methods

Compound. 4-aminoquinaldinium dodecyl acetate (I) (Caldwell and others, 1961) solubility in water, 1 in 2 at 20°. In all experiments the concentration has been expressed as μg./ml. of the acetate salt.



(I)

Microbial cultures. *Staphylococcus aureus* CN 491, originally from the Wellcome collection; phage types 47, 53, 73 and 80 were obtained from the Staphylococcal Reference Laboratory, Colindale and the antibiotic resistant strains from the Luton and Dunstable Hospital; the FDA 209 strain was obtained from the National Collection of Type Cultures.

The strain of *Streptococcus viridans* was isolated at St. Bartholomew's Hospital; the strains of *Str. agalactiae* and *Dermatophilus dermatonomus* were received from the M.A.F.F. station at Weybridge. The strain of *Proteus vulgaris* (LH.14) was originally isolated at the London Hospital;

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the strains of *Escherichia coli* and *Bacillus subtilis* were isolated in the Bacteriological Department of Allen & Hanburys Limited. The origins and sources of all other bacterial strains used are indicated in Table I.

The fungal strains were originally from St. John's Hospital for Diseases of the Skin, except *Microsporum canis*, which was supplied by Glaxo Laboratories and *Saccharomyces cerevisiae*, which was obtained from the Distillers Company Limited. The culture of *Trichomonas vaginalis* was obtained from St. Thomas's Hospital.

TABLE I

INHIBITORY ACTION OF LAUROLINIUM ACETATE *in vitro*

Micro-organism	Strain	Geometric mean M.I.C. μg./ml. at 24 hr.*
Gram-positive bacteria		
Bacillus megatherium	KM	1·7
B. subtilis		0·2
Clostridium welchii	NCTC.8237	2·5 (48 hr.)
Corynebacterium ovis	S153	6·2
Diplococcus pneumoniae	NCTC.7465	1·5
Lactobacillus acidophilus	NCTC.2949	2·2
L. brevis	NIRD.23	6·25
Sarcina lutea	NCTC.611	0·1
Staphylococcus aureus	CN.491	0·15
Staph. aureus	phage types: 47, 53, 73, 80	0·1–0·4
Staph. aureus (antibiotic resistant)	12544,† 13588,§ 12655††	0·15–0·6
Staph. saprophyticus	NCTC.7292	0·2
Streptococcus agalactiae		0·4
Str. faecalis	R(H)	1·5
Str. pyogenes	Group C CN.771	3·1
Str. viridans	Eden	0·35
Gram-negative bacteria		
Brucella abortus	S19	3·1 (48 hr.)
Escherichia coli		8·8
E. coli	NCIB.8242	6·2
Haemophilus influenzae	NCTC.8468	12·5
Klebsiella pneumoniae	NCTC.8892	17·7
Moraxella bovis	NCTC.9425	6·2
Proteus vulgaris	LH.14	>100
Pseudomonas pyocyanea	NCTC.8203	50
Salmonella dublin	98	8·8
Salmonella typhi	NCTC.786	1·56
S. typhimurium	305	12·5
Shigella flexneri	NCTC.4832	1·56
Actinomycetes		
Actinomyces bovis	NCTC.4500	>50
Dermatophilus dermatonomus	V 2020 C	0·5
Fungi and Yeast-like fungi		
Candida albicans	1549	1·25 (3 days)
Microsporum audouini	D620	0·5 (7 days)
M. canis	764E	6·25 (7 days)
Saccharomyces cerevisiae		8·8 (3 days)
Trichophyton mentagrophytes		5·0
Protozoa		
Trichomonas vaginalis		15·0 (48 hr.)
Mycoplasma hominis (PPLO)	H32515	50

† Resistant to benzylpenicillin 5 units, chloramphenicol 50 μg., tetracycline 50 μg.
†† Resistant to benzylpenicillin 5 units, streptomycin 25 μg.
§ Resistant to benzylpenicillin 5 units, streptomycin 25 μg., tetracycline 50 μg.
* Other times of incubation are shown in brackets.

TESTS FOR BACTERIOSTATIC AND FUNGISTATIC ACTIVITY

The growth inhibitory activity of laurolinium acetate was determined *in vitro* against bacterial and fungal species using the tube dilution methods described previously (Babbs and others, 1956; Collier and others, 1959; Caldwell and others, 1961). The culture medium used for the antibacterial studies was dextrose-peptone water (0·5 per cent dextrose, 1·0

per cent Bacto peptone and 0·5 per cent sodium chloride in distilled water; pH 7·2), this was supplemented with 10 per cent horse serum for the culture of *Str. agalactiae*, *Str. pyogenes*, *Moraxella bovis* and *Actinomyces bovis*. *Dermatophilus dermatonomus* was cultured in nutrient broth, and in the fungistatic experiments, the dermatophytes, *Candida albicans* and *Saccharomyces cerevisiae* were grown in Sabouraud's broth.

Trichomonas vaginalis was cultured in a modified Feinberg's medium; after inoculation with a 2 day old culture of the organism, serial dilutions of laurolinium acetate in media were incubated at 37° for 48 hr. The minimal inhibitory concentration (M.I.C.) was determined microscopically.

Tests for Bactericidal Activity

General method. The bactericidal action of laurolinium acetate was investigated on washed bacterial cell suspensions at 20° using the technique described by Caldwell and others (1961).

Modified Weber and Black method. Laurolinium acetate was also examined for its bactericidal potency using the method of Chambers (1956). Its activity at 200 μg./ml. in distilled water and in synthetic hard water (200, 400 and 600 p.p.m. $CaCO_3$), was determined against heavy suspensions of *Staph. aureus* FDA 209 and *E. coli* ATCC 11229. The percentage reduction in survival of the bacteria was estimated after drug-bacteria contact times of 30 sec. and 1 min.

Results

Antimicrobial Action in vitro

The results of growth inhibitory experiments are summarised in Table I and bactericidal activity in Tables II-IV.

TABLE II

Bactericidal activity of laurolinium acetate against representative gram-positive and gram-negative bacteria

Log. Nos. of viable organisms/ml. after exposure to 90 μg./ml. for 2·5 min. at 20° C. compared with controls.

Micro-organism	Viable count		Reduction in viability per cent
	Control	Laurolinium	
Sarcina lutea	7·36	3·30	99·991
Staphylococcus aureus (CN.491)	8·00	2·65	99·999
Staph. saprophyticus	8·10	1·70	99·999
Streptococcus viridans	7·90	5·30	99·700
Escherichia coli (lab. strain)	8·23	4·70	99·960
Proteus vulgaris	8·53	4·40	99·992
Pseudomonas pyocyanea	8·25	3·10	99·999

Antagonism studies. The results of exploring possible antagonists of laurolinium acetate are expressed in Table V.

The inactivation of the compound by serum occurs as a result of the combination with serum proteins. Using 0·1 to 2·0 per cent solutions in the presence of 50 per cent serum, the laurolinium acetate-protein precipitate formed has been separated from the supernatant liquid, and both fractions found to have antibacterial activity; that of the protein

precipitate increases at the higher concentrations of the compound studied (1·0 and 2·0 per cent) where it appears that laurolinium acetate is only loosely bound to the protein.

TABLE III

BACTERICIDAL ACTIVITY OF LAUROLINIUM ACETATE AGAINST PHAGE TYPED STRAINS OF *Staphylococcus aureus** COMMONLY ASSOCIATED WITH ANTIBIOTIC RESISTANCE

Culture strain of *Staphylococcus aureus*	Concentration of laurolinium, μg./ml.	Contact time, min.	Reduction in No. of viable bacteria, per cent
CN.491	20	2·5	99·999
	80	2·5	>99·999
	100	0·5	>99·999
		1·0	>99·999
		5·0	>99·999
Phage type 47	20	2·5	99·999
59.12867	80	2·5	100
Phage type 53	20	2·5	99·999
59.13085	80	2·5	100
Phage type 73	20	2·5	99·999
59.13241	80	2·5	100
Phage type 80	20	2·5	99·998
59.13218	80	2·5	99·999

* Bacterial suspension 10^8–10^9 micro-organisms/ml.

Therapeutic Action

Antibacterial action in vivo. Laurolinium acetate was examined for its therapeutic action in mice infected intraperitoneally with a lethal inoculum of *Staph. aureus* 663. Doses of 1, 2, 4 and 8 mg./kg., injected intraperitoneally 30 min. after the bacterial culture, failed to show any protective effects; it was not possible to examine the effects of higher doses because of their toxicity.

TABLE IV

BACTERICIDAL ACTION OF LAUROLINIUM ACETATE EVALUATED BY CHAMBERS MODIFICATION OF THE WEBER AND BLACK TEST

Micro-organism	Contact time, sec.	Reduction in No. of viable bacteria per cent by laurolinium 200 μg./ml. Water hardness (p.p.m. $CaCO_3$)			
		0	200	400	600
Staphylococcus aureus FDA.209	30	99·999	99·999	99·999	99·970
	60	99·999	99·999	99·999	99·980
Escherichia coli ATCC.11229 ..	30	99·999	99·999	99·970	98·000
	60	99·999	99·996	99·970	99·700

Sterilisation of human skin. Areas of approximately 4 cm.^{2} were marked on the inner fore-arms; in each volunteer one such area was used as a control and was swabbed with culture medium (glucose-nutrient broth with 10 per cent horse serum and 2 per cent Lubrol W added); the other skin areas were treated with aqueous and ethanolic solutions of laurolinium acetate and also ethanol alone as a further control. After drying for 2 min. the treated skin areas were swabbed with the sterile broth; swabs were incubated in the culture medium for 48 hr. at 37°. Table VI summarises the results.

Laurolinium acetate shows good activity as a skin antiseptic, both in aqueous and in 70 per cent ethanolic solution. Concentrations of 1 per cent are active against the normal bacterial flora of the skin, and of 5 per cent produce efficient sterilisation except against saprophytic spore bearing bacteria.

TABLE V

ANTAGONISM OF THE BACTERIOSTATIC ACTION OF LAUROLINIUM ACETATE AGAINST *Staphylococcus aureus* CN.491

Antagonist	Concentration, per cent	Laurolinium. Geometric mean M.I.C. in μg./ml. at 24 hr.
None		0·14
Horse serum	1	0·39
	10	6·25
	50	12·5
Saliva	10	0·19
Milk	1	1·56
	10	12·5
Pus	1	6·25
	10	100
Lecithin	0·1	50
	1	>100
	2	>100
Oxgall	1	100
	2	>100
Lubrol W	1	50
	2	100
Starch (soluble)	1	0·15
	5	0·39
	10	25
Talc	10	125
Agar	0·5	0·16
	1	1·6
	2	2·2
Liquid soap	0·01	0·32
Teepol	0·01–10	<0·14
Sodium stearate	0·01	50
	0·1	>100
Sodium palmitate	0·01	>100
	0·1	>100

Similar experiments showed that human skin areas, previously contaminated with cultures of *Proteus vulgaris* and *Pseudomonas pyocyanea* were effectively sterilised by 1 and 5 per cent aqueous solutions (Table VII).

Laurodin in 5 per cent aqueous solution did not produce any skin sensitivity, allergy or photosensitisation when applied twice daily for two weeks to shaven areas of human fore-arm and rabbit skin, nor was there

TABLE VI

ANTISEPTIC ACTIVITY OF LAUROLINIUM ACETATE AGAINST NORMAL HUMAN SKIN FLORA

Solutions	No. of positive swab cultures after incubation at 37° C. for 48 hr., expressed as the ratio of the total No. of swabs taken	
	Ratio of No. of positive swabs to total No. of swabs	Ratio of No. of positive swabs to total No. of swabs excluding aerobic spore formers
Control	1·0	0·89
Laurolinium in aqueous solution—1 per cent	0·68	0·36
5 per cent	0·52	0·09
70 per cent ethanol	1·0	0·83
Laurolinium in 70 per cent ethanol—1 per cent	0·66	0·33
5 per cent	0·14	0

any pain or smarting when the solution was applied to small cuts, abrasions and burns.

Both 1 and 5 per cent solutions were also applied twice daily for one week to wounds made by a scalpel incision (1 cm. long and 0·25 cm. deep), and to lacerations (1 cm. square) made by rubbing with coarse grade sandpaper, on the shaven flanks of guinea-pigs. The 5 per cent solution slightly delayed the healing of the scalpel wounds but did not adversely affect the healing of the lacerations; the 1 per cent solution had no delaying effect.

TABLE VII

ANTISEPTIC ACTIVITY OF LAUROLINIUM ACETATE ON HUMAN SKIN AREAS PREVIOUSLY CONTAMINATED WITH PATHOGENIC BACTERIA

Solutions	No. of positive cultures of the pathogenic strains after incubation at 37° C. for 48 hr.	
	Proteus vulgaris treated areas	*Pseudomonas pyocyanea* treated areas
Control	6/6	6/6
Laurolinium in aqueous solution—5 per cent ..	1/6	1/6
1 per cent ..	0/6	0/6
0·5 per cent ..	1/6	3/6

Adsorption on human hair. Samples of human hair (5 mg.) in aqueous solutions of laurolinium acetate (0·1, 1·0 and 5·0 per cent) for various times, were removed, blotted and rinsed once in sterile distilled water, and placed in peptone water for 30 min. The amount of the compound released was assayed microbiologically using *Staph. aureus* CN 491.

TABLE VIII

REVERSIBLE ADSORPTION OF LAUROLINIUM ACETATE BY HUMAN HAIR*

Laurolinium concentration, per cent	Laurolinium-hair contact time, min.	Laurolinium reversibly absorbed μg./mg. hair
0·1	10	1·8
	30	1·9
	60	0·9
1·0	10	1·0
	30	0·6
	60	0·5
5·0	10	0·9
	30	0·4
	60	0·3

* Laurolinium assayed microbiologically using *Staph. aureus* CN.491 as the test organism.

Appreciable concentrations of the compound are reversibly adsorbed on to human hair (Table VIII); the maximum adsorption taking place from the most dilute solution (0·1 per cent) after 10 min. contact. Higher concentrations and contact times beyond 10 min. did not result in increased adsorption.

Toxicology and General Pharmacology

Quaternary ammonium compounds are noted for their diversity of biological action; a study was therefore made of the toxicology and general

pharmacology of laurolinium acetate to see if some indication of the cause of its high parenteral toxicity could be found.

Toxicity. In acute studies in mice, the LD50 values (mg./kg.) were: oral 131·8 ± 36·2; subcutaneous 30·2 ± 5·6; intraperitoneal 2·3 ± 0·2 and intravenous 6·0 ± 0·4; mice showed marked symptoms of central nervous system depression after the intravenous injection of toxic doses. Given orally to rats in sub-acute tests, it was not toxic at a dose of 8 mg./kg. daily for 6 weeks; doses of 20 and 50 mg./kg. over the same period were toxic although blood examination showed no difference between treated and control animals.

Solutions of laurolinium acetate, in sterile distilled water (saline caused precipitation), were examined for their irritant action in the rabbit eye; 0·01 per cent was non-irritant when instilled into the eye, twice daily for 1 week; 0·1 per cent produced slight irritation and 1·0 and 5·0 per cent produced severe irritation with obvious distress to the animals.

Pharmacology. Laurolinium acetate, in doses extending to toxic levels, was devoid of any neuromuscular blocking activity, either by a curare-like or a depolarising action, nor was there any evidence of ganglionic blocking, anticholinesterase or antihistaminic activity. When injected intravenously, it had no direct effect on cardiac rhythm; however, it produced transient hypotension followed by prolonged hypertension. Large intravenous doses produced salivation, lachrymation and increased tracheal mucous secretion in the cat; these effects were not antagonised by pretreatment with atropine. In *in vitro* studies on isolated tissues, high doses appeared to be cytotoxic.

Discussion

Laurolinium acetate shows a wide *in vitro* antimicrobial activity; it is effective against representative Gram-positive and Gram-negative bacteria, against many pathogenic fungi and *Trichomonas vaginalis* and *Mycoplasma hominis* ('PPLOS').

Caldwell and others (1961) have demonstrated it to be one of the most active members of its chemical series. A comparison between the activity of this quaternary and the allied decamethylene-bis-4-aminoquinaldinium acetate (dequalinium) (Babbs and others, 1956), which has proved highly successful as a topical anti-infective agent (Wilkinson, 1959), has shown a similar range of bacteriostatic activity against Gram-positive bacteria and a closely related, although slightly lower activity against Gram-negative bacteria; the antifungal activity of the two agents is also very similar. The significant difference between the mono and bis quaternary ammonium compound is that unlike dequalinium, laurolinium acetate has, in addition to its other properties, a rapid and potent bactericidal activity which is more pronounced against Gram-positive bacteria.

With *Staphylococcus aureus*, the bacteriostatic and bactericidal activity of laurolinium acetate is independent of strain differences; thus it is equally effective against the laboratory strain of *Staph. aureus* CN 491,

the phage types 47, 53, 73 and 80, and against strains resistant to benzylpenicillin and other antibiotics. This lack of strain specificity in its action is good supportive evidence that, like other cationic antibacterial agents, it combines with specific anionic sites in the bacterial cell.

The efficacy of laurolinium acetate as a bactericide in Chambers modification of the Weber and Black test (Chambers, 1956) is well shown by the almost complete sterilisation (99·999 per cent) of cultures of *Staph. aureus* and *E. coli*, by a concentration of 200 μg./ml. in contact for 30 sec. Bactericidal activity decreases in the presence of increasing water hardness although this is more evident against *E. coli* than against *Staph. aureus*. This method of evaluating cationic bactericides, although not common in this country, is included in the recommended methods of the United States Public Health Service and the U.S. Department of Agriculture.

As with most quaternary ammonium compounds, its antibacterial action is antagonised by organic matter and by anionic substances. The antagonism shown by the soaps (but not potash soft soap) and bile salts is common to many cationic bactericides. It is not antagonised to any great extent by saliva, but there is some antagonism by milk, pus and serum. Serum antagonism is due to precipitation of the serum proteins; however, the compound is only loosely bound to the precipitate. In the presence of a slight excess of laurolinium acetate the compound-protein precipitate retains antibacterial activity. In similar studies Van Eseltine and Hucker (1948) showed that a closely related quaternary salt—laurylisoquinolinium bromide was less antagonised in its antibacterial action in the presence of serum, milk, cotton-seed-oil and soluble starch, than were a number of other aliphatic quaternary ammonium compounds in use at that time.

The rapid bactericidal activity of laurolinium acetate in *in vitro* experiments suggested that solutions of the compound might be used to sterilise the skin and its appendages. In subsequent tests on human volunteers swabbing the fore-arm with 5·0 and 1·0 per cent solutions in water or 70 per cent ethanol reduced the normal skin flora. Related studies to examine the activity of solutions on intact skin areas, previously contaminated with cultures of *Proteus vulgaris* and *Pseudomonas pyocyanea*, also showed its sterilising ability. The results from adsorption studies using human hair showed that the compound was well adsorbed over a wide range of concentrations; and local toxicity studies showed it to be free from untoward effects on intact and abrased skin, even though repeated applications of a 5 per cent solution were made. In the clinic, Verdon (1961) has made a comparative survey of the efficacy of a series of common skin antiseptics under ward conditions; the results of which so favoured the use of a 5 per cent solution of laurolinium acetate that this has since been adopted as the standard pre-injection skin disinfectant in all wards of the Portsmouth Group of Hospitals.

From the present study it is apparent that laurolinium acetate is suitable for use in the treatment of local infective conditions, for the sterilisation of skin areas and for general antiseptic purposes. Unfortunately its toxicity precludes its systemic use and its basic pharmacology

suggests that the systemic toxicity is related to protein precipitating activity.

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