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NEW PLASMID CURING COMPOUNDS. ANTHRIL AND PHENATHRIL DERIVATIVES

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Abstract—The plasmid curing activities of some newly synthesized tricyclic compounds has been studied. Anthracene was substituted at the 1st, 2nd or 9th carbon atom with acetyl-, dimethylamino-propanone, dimethylaminpropanol or dimethylaminopropene radicals. Derivatives of phenathrene similarly substituted at the 9-postition were also synthesized.

All the dimethyl-aminopropanol substituted anthril- and phenanthril-derivatives showed significant curing effects on the *E. coli* K12 strain carrying an *F'lac*-plasmid. The most effective compounds were the symmetrically substituted 9-anthril- and 9-phenanthril derivatives producing 25-40 per cent cured clones under the standard conditions. Disruption of the π-electron system of the three rings caused a significant decrease in the plasmid curing effect of 9-substituted anthracene.

Transferable drug resistance factors or other plasmids in bacteria can be eliminated by various heterocyclic compunds. Acridine orange is an effective curing agent which is able to eliminate F'-plasmids [1] or R-plasmids of *E. coli* [2]. Another heterocyclic compound, ethidium bromide (a trypanocidal drug) has also been found to be a plasmid curing agent in enterobacteria and staphylcocci [3].

We have previously reported that several tricyclic drugs, chlorpromazine, promethazine, levomepromazine, imipramine etc. used in various psychiatric disorders are also effective as antibacterial and plasmid curing agents *in vitro* [4–7]. These compounds, together with acridine and ethidium have in common a tricyclic ring structure with one or two heteroatoms in the central ring. Thus the nucleophylic-charge and charge position of the heteroatoms in the tricyclic ring system may play an important role in the antibacterial and plasmid curing activity of the above compounds.

In the present experiments different acridine and ethidium carbocyclic analogues without any heteroatoms in the ring system, as well as some of their anthril- and phenanthril-derivatives carrying substituent side chains at different parts of the ring system, were tested for plasmid curing activity. The anthril- and phenanthril-derivatives, which were synthesized in our laboratory, included substituent cationic side chains bound to the ring system either symmetrically (9-anthril-derivatives) or asymmetrically (1-anthril, 2-anthril- and 9-phenanthril- derivatives).

MATERIALS AND METHODS

Bacterial strain. E. coli K12 LE 140 (*str*®, *lac*$^{-}$Δ, *mal*$^{-}$, λ^{r}, F'*lac*$^{+}$). This strain is *lac*$^{-}$ on the chromosome but carries an F-prime plasmid which contains the *lac* region.

Chemicals. 1-acetylanthracene, 1-dimethylamino-3-(1-anthril)-3-propanone, 1-dimethylamino-3-(1-anthril-3-propanol, 1-dimethylamino-3-(1-anthril)-2-propene (Fig. 1a).

2-acetylanthracene, 1-dimethylamino-3-(2-anthril)-3-propanone 1-dimethylamino-3-(2-anthril)-3-propanol (Fig. 1b). 9-acetylanthracene, 1-dimethylamino-3-(9-anthril)-3-propanone, 1-dimethylamino-3-(9-anthril)-3-propanol, 1-dimethylamino-3-[9-(10-hydro)-anthril]-3-propene 1-dimethylamino-3-[9(9,10-dihydro)-anthril]-3-propanol (Fig. 1c). 9-acetylphenanthrene, 1-dimethylamino-3-(9-phenanthril)-3-propanone, 1-dimethylamino-3-(9-phenanthril)-3-propanol, diethylaminomethyl-α-methyl-9-phenanthrene 1-diethylamino-methyl-9-phenanthrene (Fig. 1d). The amino-cetone derivatives were synthesized via Mannich-condensation from the corresponding acetyl-compounds and the amino-alcohols from the Mannich-base through reduction with sodium tetrahydroborate.

Media. The antibacterial and plasmid curing effect of the tested compounds were studied in modified MTY liquid media [8], containing 1.0 g NH_4CL; 7.0 g K_2HPO_4; 3.0 g NaH_2PO_4; 0.8 g NaCl; 1.0 g D-glucose; 10.0 g Bacto tryptone (Difco) and 1.0 g yeast extract (Difco) in 1.0 litre distilled water at pH 7.2. For plating the bacteria eosine–methylene-blue (EMB) agar [9] was used, in order to distinguish *lac*$^{+}$ colonies from those formed by *lac*$^{-}$, plasmidless bacteria

The study of antibacterial effects. An overnight preculture of *E. coli* K12 LE 140 was diluted 10^4-fold and 0.1 ml (ca. 5×10^3 bacteria) was inoculated into 5.0 ml of MTY broth containing various concentrations of the different compounds. The cultures were grown at 37° without shaking. Minimum inhibitory concentrations were read after 24 hr incubation.

The elimination of F'lac *plasmid.* Plasmid curing was carried out with the method described previously [6]. The overnight preculture of *E. coli* K12 LE 140 was

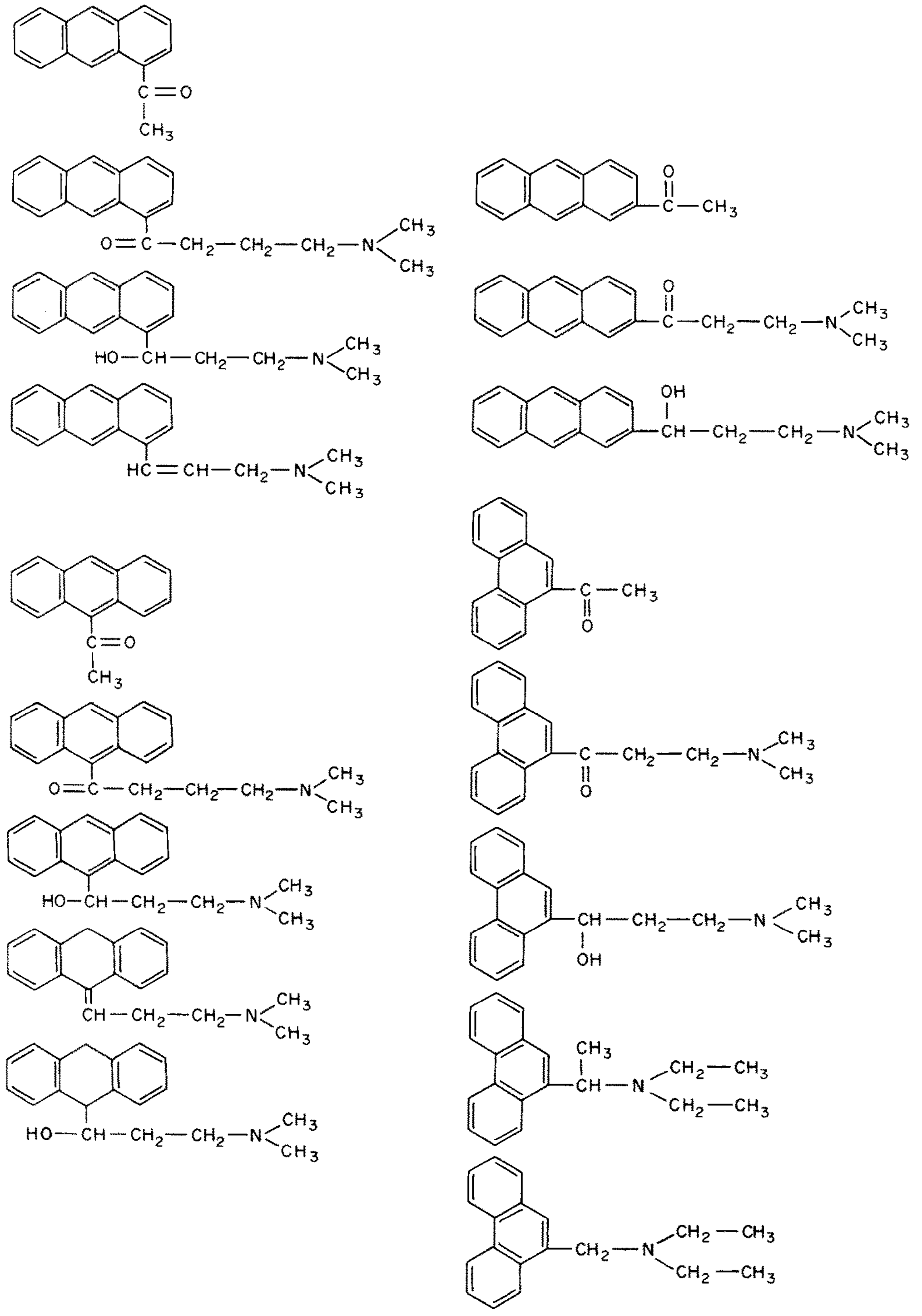

Fig. 1. Chemical structure of compounds used. Compounds are listed in the same order as in Table 1.

diluted to 10^3 bacteria/ml in MTY broth. The tested compounds were added to 5.0 ml of the diluted culture at different final concentrations (0–100 μg/ml). The cultures were incubated at 37° without shaking. After 24 hr incubation the cultures were diluted in isotonic saline and plated to obtain individual colonies on eosine methylene-blue agar. The plates were incubated at 37° for 24 hr then the *lac*$^+$ and *lac*$^-$ colonies were counted separately.

RESULTS

Anti-bacterial activity of anthril- and phenanthril-derivatives

The anthril-derivatives were divided into three groups according to whether the substituents were bound to the 1st, 2nd or 9th carbon atom of the ring system. Each of the three groups of anthril-derivatives and the 9-phenanthril-derivatives also contained

Table 1. The bacteriostatic effect of different compounds on strain *E. coli* K12 LE-140

Compounds	Minimum inhibitory concentrations μg/ml
1 1-acetylanthracene	50
2 1-dimethylamino-3-(1-anthril)-3-propanone	400
3 1-dimethylamino-3-(1-anthril)-3-propanol	200
4 1-dimethylamino-3-(1-anthril)-3-propene	20
1 2-acetylanthracene	60
2 1-dimethylamino-3-(2-anthril)-3-propanone	1000
3 1-dimethylamino-3-(2-anthril)-3-propanol	300
1 9-acetylanthracene	70
2 1-dimethylamino-3-(9-anthril)-3-propanone	1000
3 1-dimethylamino-3-(9-anthril)-3-propanol	900
4 1-dimethylamino-3-[9-(10-hydro)-anthril]-3-propene	400
5 1-dimethylamino-3-[9-(9,10-dihydro-)-anthril]-3-propanol	1000
1 9-acetylphenanthrene	100
2 1-dimethylamino- 3-(9-phenanthril)-3-propanone	500
3 1-dimethylamino-3-(9-phenanthril)-3-propanol	110
4 1-diaethylamino-methyl-α-methyl-9-phenanthrene	220
5 1-diaethylamino-methyl-9-phenanthrene	260

various substituents in the side chain (see Fig. 1a–d). In each case, the minimal inhibitory bacteriostatic concentration (MIC) of the derivatives was determined prior to the plasmid curing experiments. The MIC values are summarized in Table 1.

The minimal bacteriostatic concentrations varied according to the substituents groups, although in the great majority of cases introduction of the dimethylamino side chain led to an increase in MIC. The most effective antibacterial agent was 1-dimethylamino-3-(1-anthril-)2-propene , whilst 1-dimethylamino-3-propanone substituents were all virtually ineffective. There was no correlation between antibacterial activity and plasmid curing effects of the tested compounds.

Curing activity of synthesized compounds

1. 1-*anthril-derivatives.* In plasmid curing experiments with 1-anthril-derivatives 40 μg/ml 1-acetyl anthracene was found to eliminate the F′*lac* plasmid with a frequency of 0.2 per cent; 1-dimethylamino-3-(1-anthril)-3-propanone had no curing effect even at a concentration of 200 μg/ml, whilst, in contrast, the 3-propanol derivative produced up to 5 per cent

Table 2. The elimination of F′*lac* plasmid by anthril-derivatives

(a) 1-dimethylamino-3-(1-anthril)-3-propanol

Concentration μg/ml	lac^{-}* colonies %	Viable cells after 24 hr	No. of colonies tested
0	0	6.0×10^8	2121
50	0	4.5×10^8	200
80	0.1	3.2×10^8	1420
100	0.5	3.0×10^8	846
120	1	1.0×10^8	970
140	5	7.0×10^7	984
160	5	2.0×10^6	625

(b) 1-dimethylamino-3-(9-anthril)-3-propanol

Concentration μg/ml	lac^{-}* colonies %	Viable cells after 24 hr	No. of colonies tested
0	0	5.0×10^8	942
400	0	2.4×10^7	2600
500	2	1.4×10^7	1540
600	24	2.0×10^7	1100
700	3	9.0×10^6	2200
800	2	3.0×10^6	1570
900	0	2.0×10^5	2460
1000	—	—	—

**E. coli* LE140 was grown overnight in the presence of the indicated concentrations of drug and plated on EMB-lactose agar (see Methods) in order to determine the number of cured (i.e. lac^-) clones)

Table 3. The elmination of F′*lac* plamid by phenanthril-derivatives

(a) 1-dimethylamino-3-(9-phenanthril)-3-propanol

Concentration μg/ml	colonies %	lac^- Viable cells after 24 hr	No. of colonies tested
0	0	6.0×10^8	871
20	0	4.0×10^8	940
40	1	4.0×10^8	1825
60	5	2.0×10^8	2540
80	40	2.1×10^7	1796

(b) diaethylamino-methyl-α-methyl-9-phenanthrene

Concentration μg/ml	colonies %	lac^- Viable cells after 24 hr	No. of colonies tested
0	0	6.4×10^8	837
50	0.3	6.0×10^8	2015
100	4.0	3.3×10^8	1980
120	5.0	1.3×10^8	1746
140	6.0	7.6×10^7	1416
160	12.0	4.0×10^7	1295
180	26.0	1.7×10^7	1840
200	—	—	—

(c) 1-dimethylamino-methyl-9-phenanthrene

Concentration μg/ml	colonies %	lac^- Viable cells after 24 hr	No. of colonies tested
0	0	3.5×10^8	1560
0	0	7.4×10^7	1200
140	1.0	3.9×10^7	1600
160	2.0	3.7×10^7	3700
180	0.5	5.1×10^6	1100
200	0.1	2.6×10^4	2000

curing of the bacteria (Table 2). Finally, 1-dimethylamino-3-(1-anthril)-2- propene was without effect over the concentration range of 14–20 μg/ml despite its very low MIC value (20 μg/ml).

2 2-*anthril derivatives.* 2-acetyl-anthracene eliminated the plasmid with a rather low (0.1 %) frequency at a concentration of 40 μg/ml; 1-dimethyl-3-(2-anthril)-3-propanone had no curing effect even at 400 μg/ml concentration and only a low efficiency of curing (0.1–0.2 per cent at 200 μg/ml) was obtained with the 3-propanol derivative of 1-dimethylamino-3-(2-anthril). Thus the 2-anthril-derivatives all showed very weak curing effects.

3. 9-*anthril derivatives.* 9-acetyl-anthracene produced 0.5 per cent of cured clones at a concentration of 40 μg/ml, whilst 1-dimethylamino-3-(9-anthril)-3-propanone was ineffective even at 200–400 μg/ml. However, the 3-propanol derivative in this case eliminated the plasmid with relatively high frequency at concentrations of 500–600 μg/ml (Table 2).

When the conjugation of the π-electrons is broken in the L-region of the ring system, the plasmid curing effect disappears. Thus, 1-dimethylamino-3-[9(9,10-dihydro)-anthril]-3-propanol [see Fig. 1c(v)] failed to eliminate the F′*lac* plasmid even at a concentration of 400 μg/ml.

4. 9-*phenanthrene derivatives.* In the presence of 20 and 80 μg/ml 9-acetyl-phenanthrene, the efficiency of curing was 0.1 and 0.25 per cent respectively whilst 400 μg/ml of 1-dimethylamino-3-(9-phenanthril)-3-propanone was ineffective. The 3-propanol derivative was again an efficient curing agent producing up to 40 per cent of cured clones (Table 3). When diaethylamino-methyl-α-methyl-9-phenanthrene [Fig. 1d(iv)] was used, efficient elimination of the plasmid was also obtained (Table 3), but interestingly the absence of the α-methyl group in 1-diethylamino-methyl-9-phenanthrene led to reduced curing (Table 3). Finally, it should be noted that the sensitivity of plasmidless lac^- bacteria to all the compounds tested was the same as that of the lac^+ strain.

DISCUSSION

We present here evidence that some new tricyclic compounds tested, i.e. anthril- and phenanthril-derivatives which do not contain a heteroatom in the ring system, are able to eliminate an F′*lac* plasmid of *E. coli.* Anthracene and phenanthracene were substituted at various positions with cationic side chains and a symmetrical dimethylamino-propanol group was found to have highest activity. Among the anthril-derivatives the 9-anthril was the most effective and the 2-anthril-derivatives showed the least activity. The plasmid curing effect of the 1-anthril-derivatives was intermediate to that of the 9- and 2-anthril-derivatives. The data indicate the particular importance of the symmetry of the various substituents of 1-, 2-, and 9-acetyl-anthracene. With the dimethylamino-propanone substitution of both anthril- and phenanthril-derivatives the compounds had virtually no plasmid curing action. These derivatives also had the least bacteriostatic effects.

The reason for the ineffectiveness of compounds with the dimethylamino-3-propanone side chain may be due to the strong electronegative oxygen atom on the 3rd carbon of the propil side chain being able to polarize the π-electron system of the three rings. The importance of charge symmetry was also shown in other experiments. Acridine orange is an effective curing agent, whilst rivanol, having three asymmetric substituents on the acridine molecule, shows very small curing effects [10]. We suggest that the ineffectiveness of methylene blue [10–12] might also be due to charge asymmetry.

Bacterial plasmids are eliminated by different mechanisms. The intercalation of acridine dyes into DNA probably differentially inhibits the replication of plasmid DNA [11, 13], whilst sodium dodecylsulphate (SDS) eliminates plasmid bearing cells by a selection mechansim [14]. Plasmid bearing bacteria and lac^-, plasmidless bacteria showed the same sensitivity to the compounds used in this study indicating that curing did not involve a selective mechanism. Moreover, both of the effective anthril- and phenanthril-derivatives interact with DNA *in vitro* (unpublished results). As an alternative to plasmid elimination, following intercalation of a drug into DNA, binding to the outside of the DNA molecule or to a protein component of the replication machinery might also be effective as has been suggested for acridine dyes [15]; phenanthridines [16]; tilorone [17] and chlorpromazine [18]. At the moment, we are unable to distinguish between intercalation into DNA or direct action on the replication complex as the mechanism of curing by anthril- and phenanthril-derivatives.

It seems likely that the molecular orbitals, the charge and the conformation of the substitiuents may play some role in the plasmid curing action of tricyclic compounds. We suppose that the planar carbocyclic three-ring system with conjugated π-electrons (e.g. acridine orange, ethidium, phenothiazines, and the newly synthesized anthril- and phenanthril-derivaties), are able to eliminate bacterial plasmids when appropriate groups are substituted in the molecule. The substituent appears most effective when symmetrically bound to the ring system. The quality of the side chain also appears important, particularly the cationic property, e.g. the tertiary amino group representing the positive-pole of a well-determined molecule orbital.

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