Clofibrate administered orally 1 h before pylorus ligation antagonized the induced ulcer formation with the ED50 being 430 mg kg^{-1} (Fig. 1C). Clofibric acid also exhibited the activity being similar in potency (ED50: 340 mg kg^{-1}; 1·28 (0·95 to 1·87)).

The present findings demonstrate that clofibrate is an effective inhibitor of gastric acid secretion and ulcer formation in the rat. In comparison, clofibric acid administered orally exhibited essentially the same activities thus indicating the activities are due to the free acid form of the drug. Such a suggestion would be consistent with the observation that the free acid is rapidly formed from the ester (Thorp, 1962). Clofibrate, and the free acid, appears to be a potentially useful therapeutic agent for the treatment of hypergastric acid secretion and peptic ulcers.

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REFERENCES

FINNEY, D. J. (1964). *Statistical Method in Biological Assay*, New York: Hafner Co.

LIPPMANN, W. (1970). *J. Pharm. Pharmac.*, **22**, 568–573.

LIPPMANN, W. & SEETHALER, K. (1973). *Experientia*, **29**, 993–995.

OLIVER, M. F. (1971). *Br. med. J.*, **4**, 767–775.

SHAY, H., SUN, D. C. H. & GRUENSTEIN, M. (1954). *Gastroenterology*, **26**, 906–913.

SHAY, H., KOMAROV, S. A., FELS, S. S., MERANZE, D., GRUENSTEIN, M. & SIPLET, H. (1945). *Ibid.*, **5**, 43–61.

THORP, J. M. (1962). *Lancet*, **1**, 1323–1326.

THORP, J. M. & WARING, W. S. (1962). *Nature (Lond.)*, **194**, 948–949.

Evaluation of the binding of some substituted anthraquinones and naphthacenequinones to DNA

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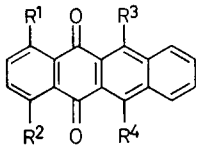
We have previously reported the interactions of a series of substituted anthraquinones with DNA (Double & Brown, 1975). These anthraquinone derivatives were designed to incorporate features known to be essential for binding to DNA and were found to have affinity constants for the interaction with DNA within the range of these constants for drugs known to intercalate into DNA. All intercalating agents contain, as an essential requirement, a planar electron-rich chromophore, and binding to DNA is enhanced when there is a substituent bearing an amino group which can bind electrostatically to the phosphate groups of the DNA. The area of the planar chromophore has been calculated as 38–49 $Å^2$ for acridines and benzacridines which intercalate (Albert, 1973) and this compares favourably with a value of about 50 $Å^2$ for the area of a hydrogen-bonded base pair in the B form of DNA (Arnott, 1970). This means that it is possible for planar tricyclic and tetracyclic ring systems to be accommodated between successive base pairs. The compounds tested previously (Double & Brown, 1975) were tricyclic, and the overall effect of increasing the system to four rings cannot be predicted as two opposing forces would be expected. The increase in electron density should increase the stability of the complex and consequently the association constant, whereas the increase in size could give an inhibitory steric effect. Hence four further compounds (Ia–d), containing an equivalent tetracyclic chromophore, have been prepared to examine the effect of increasing the size of the planar ring system on the binding to DNA.

Of the compounds tested previously, the anthraquinone derivative II, 1,4-di-(4-diethylamino-1-methylbutylamino)-9,10-anthraquinone, was found to have the highest association constant for the interaction with DNA. The analogous naphthacenequinone, 1,4-di-(4-diethylamino-1-methylbutylamino)-5,12-naphthacenequinone, (Ia) was therefore prepared and to examine the effect of a reduced capacity for electrostatic attraction, the analogue Ib, 1-chloro-4-(4-diethylamino-1-methylbutylamino)-5,12-naphthacenequinone, was also prepared. The synthetic route to compounds Ia and IIb initially involved synthesis of 1,4-dichloro-5,12-naphthacenequinone (I; $R^1 = R^2 =$ Cl, $R^3 = R^4 =$ H) by the method of Waldman & Mathiowetz (1931). This was then reacted with 2-amino-5-diethylaminopentane according to Double & Brown (1975). Extraction of an acidic solution of the product with chloroform yielded the mono-substituted compound Ib which was purified by chromatography. Basification followed by extraction with chloroform yielded the disubstituted compound Ia which was also purified by chromatography. For further comparison the analogue Ic was prepared since it might be anticipated that here the additional aromatic ring would have a lesser effect on increasing the stability of the complex with DNA than for compound Ib. The chloro- precursor (6-chloro-5,12-naphthacenequinones, I; $R^1 = R^2 = R^3 =$ H, $R^4 =$ Cl) was therefore prepared from 6-hydroxy-5,12-naphthacenequinone by the method of Marschalk & Stumm (1948) and heated under reflux with 2-amino-5-diethylaminopentane. The required product Ic, 6-(4-diethylamino-1-methylbutylamino)-5,12-naphthacenequinone was isolated by standard procedures (Double & Brown, 1975).

It was expected that these tetracyclic compounds (Ia–c) would show a marked increase in hydrophobicity and so a fourth analogue (Id) was prepared in which an attempt was made to increase the polarity by incorporation of two hydroxyl functions. This was also desirable as it improved the analogy between these synthetic compounds and the antibiotics daunomycin and adriamycin upon which they have been modelled. 3-Chlorophthalic anhydride was prepared from 3-nitrophthalic anhydride (Smith, 1933) and heated with a mixture of boric acid and 1,4-dihydroxynaphthalene (Finkelstein & Romano, 1970) to yield 1-chloro-6,11-dihydroxy-5,12-naphthacenequinone (I; $R^1 =$ Cl, $R^2 =$ H, $R^3 = R^4 =$ OH). The compound Id 1-(4-diethylamino-1-methylbutylamino)-6,11-dihydroxy-5,12-naphthacenequinone was then prepared by the method used previously for compounds Ia–c. The four naphthacenequinones (Ia–d) were then prepared as their hydrochloride salts. These compounds all contain those requirements thought to be essential for the binding of daunomycin and adriamycin to DNA (Pigram, Fuller & Hamilton, 1972; Dimarco & Arcamone, 1975). A series of compounds similarly designed to mimic these antibiotics have been prepared by Müller, Flügel & Stein (1971), in which the 1,4,5-trihydroxyanthraquinone nucleus is substituted at the 2-position (for example IIIa and IIIb). We have extended this series by preparation of IIIc, 2-(4-diethylaminobenzyl)-1,4-dihydroxy-9,10-anthraquinone, and IIId 2-(4-diethylaminobenzyl)-1,8-dihydroxy-9,10-anthraquinone, by Marschalk reaction (Marschalk, Koenig & Ourousoff, 1936) of *p*-diethylaminobenzaldehyde with the leuco-derivative of 1,4-dihydroxyanthraquinone (Meyer & Sander, 1920) and with the leuco-derivative of 1,8-dihydroxyanthraquinone respectively. (The leuco-derivative of 1,8-dihydroxyanthraquinone was prepared by catalytic reduction over Raney nickel using morpholine as solvent). Compounds IIIc and IIId were prepared as their hydrochloride salts.

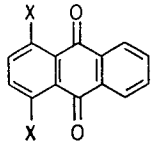
It was found that DNA was precipitated as a fibrous coloured DNA/ligand complex on addition of aliquots of DNA solution to solutions of compounds Ia–d in buffer of pH 7·0 and ionic strength 0·018 by the technique of Double & Brown (1975). This precipitation is almost certainly due to electrostatic interaction of the ligand with the anionic exterior of the DNA helix followed by stacking of the planar ligand molecules



I

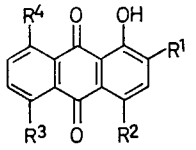
	R^1	R^2	R^3	R^4
Ia	X	X	H	H
Ib	X	Cl	H	H
Ic	H	H	X	H
Id	X	H	OH	OH

X = $NHCHMe[CH_2]_3\ NEt_2$



II

X = $NHCHMe[CH_2]_3\ NEt_2$



III

	R^1	R^2	R^3	R^4
IIIa	$CH_2CH_2{\cdot}C_6H_{10}{\cdot}NH_2(p)$	OH	OH	H
IIIb	$CH_2CH_2NMe_2$	OH	OH	H
IIIc	$CH_2{\cdot}C_6H_4{\cdot}NEt_2(p)$	OH	H	H
IIId	$CH_2{\cdot}C_6H_4{\cdot}NEt_2(p)$	H	H	OH

(Lochmann & Micheler, 1973). The association constant for this type of interaction can be higher than that for intercalation (Stone & Bradley, 1961) and this directed stacking can therefore occur in preference to other modes of binding. This precipitation with DNA emphasizes the designed increase in π electron-density, manifested as the increased potential for stacking, in this series of naphthacenequinones compared to the anthraquinones prepared previously. Since the initial directing interaction before stacking is electrostatic binding, this stacking on the outside of the helix can be prevented by increasing the ionic strength of the medium (Wakelin & Waring, 1974). The addition of DNA was therefore attempted using buffer containing 0·5 M sodium chloride. Although compound Id had been designed to have an increased hydrophilicity it still gave precipitation of the DNA. This is in fact consistent with theories that compounds with electron-rich substituents have a greater tendency to stack than their unsubstituted analogues due to an increased polarizability of the molecules as was shown to occur with purines (Ts'o, 1968). Further increase in ionic strength gave precipitation of compound Id. Compounds Ia–c did not give precipitation with DNA at the increased ionic strength. Although compound Ib is insoluble in this buffer, solubilization by the DNA occurs at low ligand to DNA ratios. These naphthacenequinone derivatives gave a decrease in their ultraviolet absorption spectra and a small red shift with an ill-defined isosbestic point on increasing the DNA concentration at a fixed concentration of ligand. This change in absorption spectrum had also been seen with the anthraquinone derivatives (Double & Brown, 1975) and is thought to be due to a change in the polarity of the environment, the drug moving from a hydrophilic to a hydrophobic environment (Laurence, 1952). To simulate this change, solutions of ligand were prepared containing $\frac{1}{2}\%$ Brij 56 (a cetomacragol surfactant at a concentration above its cmc) and similar changes in the absorption spectra were seen in accord with this postulate. The anthraquinone derivatives IIIc and IIId were found to be insoluble in both buffers and still precipitated when added

as solutions in DMSO to solutions of DNA. If they had a high affinity for DNA then solubilization should have occurred at low ligand to DNA ratios as seen with compound Ib. The continued precipitation suggests they have not a high affinity for DNA consistent with the hypothesis that an ionized amino group is essential for binding to DNA.

The naphthacenequinones Ia–c were assayed by the method of spectrophotometric titration described previously (Double & Brown, 1975) at pH 7·0 and in buffer at ionic strength 0·5. An accurate affinity for the DNA-Ib interaction could not be determined since free ligand precipitated from solution despite the DNA being added to the drug solution. The affinity constant for the interaction of Ia with DNA is $4{\cdot}04 \times 10^6$ M^{-1} and for the interaction of Ic with DNA is $4{\cdot}1 \times 10^5$. These values compare favourably with the value of $4{\cdot}3 \times 10^6$ M^{-1} obtained for the anthraquinone derivative II at an ionic strength of 0·018 since the affinity constant is known to decrease with increasing ionic strength. For example the affinity constant for the DNA/ethidium interaction decreases more than twentyfold on increasing the ionic strength from 0·2 to 0·5 (Wakelin & Waring, 1974). Hence the DNA binding of the naphthacenequinone Ia is comparable to and probably stronger than that of its tricyclic analogue, II, showing that the site will accommodate a tetracyclic structure. Since external binding has been avoided by increasing the ionic strength, the naphthacenequinone must be presumed to be binding to the bases of DNA.

This study has been concerned with the synthesis and the evaluation of the binding to DNA of compounds designed to contain those features of daunomycin thought to be essential for its interaction with DNA. The planar naphthacenequinones, whilst having a strong affinity for DNA, readily stack externally on DNA. This suggests that the study should be extended to include tetracyclic compounds in which the stacking interaction is prevented sterically and yet which still can bind to DNA. This should be feasible since daunomycin itself has these features and also it is known that for ethidium analogues quite extensive structural modifications can be made without impairing the potential for binding to DNA (Wakelin & Waring, 1974).

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REFERENCES

ALBERT, A. (1973). *Selective toxicity*, 5th Edition. London: Chapman and Hall.

ARNOTT, S. (1970). *Prog. Biophys. mol. Biol.*, **21**, 267–319.

DIMARCO, A. & ARCAMONE, F. (1975). *Arzneimittel-Forsch.*, **25**, 368–375.

DOUBLE, J. C. & BROWN, J. R. (1975). *J. Pharm. Pharmac.*, **27**, 502–507.

FINKELSTEIN, J. & ROMANO, J. A. (1970). *J. medl Chem.*, **13**, 568–570.

LAURENCE, D. J. R. (1952). *Biochem. J.*, **51**, 168–180.

LOCHMANN, E-R. & MICHELER, A. (1973). In *Physicochemical properties of nucleic acids*. Editor: Duchesne, J. London and New York: Academic Press.

MARSCHALK, C., KOENIG, F. & OUROUSOFF, N. (1936). *Bull. Soc. chim. Fr.*, 1545–1575.

MARSCHALK, C. & STUMM, C. (1948). *Ibid.*, 418–428.

MEYER, K. H. & SANDER, A. (1920). *Ann.*, **420**, 113–125.

MÜLLER, W., FLÜGEL, R. & STEIN, C. (1971). *Ibid.*, **754**, 15–27.

PIGRAM, W. J., FULLER, W. & HAMILTON, L. D. (1972). *Nature, New Biol.*, **235**, 17–19.

SMITH, J. C. (1933). *J. chem. Soc.*, 643–644.

STONE, A. L. & BRADLEY, D. F. (1961). *J. Am. chem. Soc.*, **83**, 3627–3634.

TS'O, P. O. P. (1968). In *Molecular associations in biology*. Editor: Pullmann, B. London and New York: Academic Press.

WAKELIN, L. P. G. & WARING, M. J. (1974). *Mol. Pharmac.*, **10**, 544–561.

WALDMAN, H. & MATHIOWETZ, H. (1931). *Chem. Ber.*, **64**, 1713–1724.