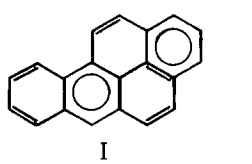
Absence of Mutagenicity of Coralyne and Related Antileukemic Agents: Structural Comparison with the Potent Carcinogen 7,12-Dimethylbenz[*a*]anthracene

C. C. CHENG*x, ROBERT R. ENGLE ‡, JOHN R. HODGSON *, ROBERT B. ING ‡, HARRY B. WOOD, Jr. ‡, SHOU-JEN YAN *, and ROBERT K. Y. ZEE-CHENG *

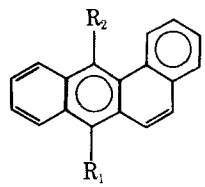
Abstract □ The structural similarity between the antileukemic alkaloid coralyne and the carcinogenic and antineoplastic hydrocarbon 7,12-dimethylbenz[*a*]anthracene, as well as the similarity between the antileukemic alkaloid nitidine and the carcinogenic hydrocarbon 5-methylchrysene, prompted a mutagenicity evaluation of coralyne sulfoacetate, nitidine chloride, the 8-ethyl homolog of coralyne, nitidine methosulfate, and the tetramethoxy analog of nitidine by the Ames method against the histidine-auxotroph strains of *Salmonella typhimurium* TA-1537, TA-1538, TA-98, and TA-100; 7,12-dimethylbenz[*a*]anthracene was used as a reference standard. The mutagenicity of these antileukemic compounds was either completely eliminated or drastically reduced, but the mutagenic response was generally high for 7,12-dimethylbenz[*a*]anthracene. The results suggest that the presence of a quaternary nitrogen atom and alkoxy groups could be important in alleviating the mutagenicity of the parent mutagenic and carcinogenic hydrocarbons.

Keyphrases □ Coralyne and related compounds—mutagenicity evaluated, compared to 7,12-dimethylbenz[*a*]anthracene □ 7,12-Dimethylbenz[*a*]anthracene—mutagenicity compared to coralyne and related compounds □ Mutagenicity—evaluated in coralyne and related compounds, compared to 7,12-dimethylbenz[*a*]anthracene □ Antileukemic agents—coralyne and related compounds, mutagenicity evaluated, compared to 7,12-dimethylbenz[*a*]anthracene □ Carcinogenic agents—7,12-dimethylbenz[*a*]anthracene, mutagenicity compared to coralyne and related compounds □ Structure-activity relationships—coralyne and related compounds, mutagenicity evaluated and compared to 7,12-dimethylbenz[*a*]anthracene

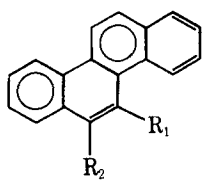
Among numerous polycyclic hydrocarbons studied, the carcinogenic activity of benzo[*a*]pyrene (I) is well documented. While two structurally related hydrocarbons, benz[*a*]anthracene (II*a*) and chrysene (III*a*), are relatively noncarcinogenic, several of their methylated derivatives, such as 7-methylbenz[*a*]anthracene (II*b*), 7,12-dimethylbenz[*a*]anthracene (II*c*), 5-methylchrysene (III*b*), and 5,6-dimethylchrysene (III*c*), exhibited strong carcinogenicity and some were no less potent than benzo[*a*]pyrene (1–6).



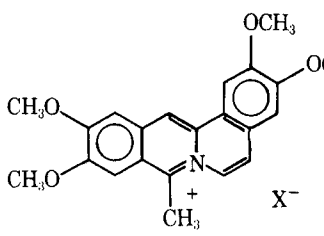
I



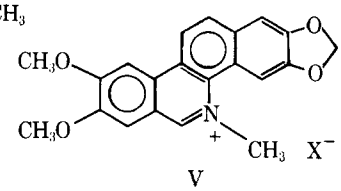
II*a*: $R_1 = R_2 = H$
II*b*: $R_1 = CH_3$, $R_2 = H$
II*c*: $R_1 = R_2 = CH_3$



III*a*: $R_1 = R_2 = H$
III*b*: $R_1 = CH_3$, $R_2 = H$
III*c*: $R_1 = R_2 = CH_3$



IV



V

DISCUSSION

Carcinogenesis of compounds of this type has been interpreted and correlated by electronic and geometric structures of these polycyclic ring systems (7–10), resulting in the postulation of K, L, and M regions at different parts of polynuclear structures as areas possibly involved in carcinogenic metabolism, noncarcinogenic metabolism, and metabolic epoxidation–perhydroxylation, respectively (2, 11). These regional assignments are by no means decisive for the explanation and prediction of carcinogenicity of polycyclic hydrocarbons, and certain aspects of the original postulation may be obsolete and may have to be modified or correlated with other factors (12–17). Nevertheless, the concept is still being used in structure-activity studies of carcinogenesis (18–23).

The antileukemic activity of coralyne (IV), nitidine (V), and related structural and positional isomers against the P-388 lymphocytic leukemia system in mice was reported (24–27). The basic structural skeleton of IV bears a striking resemblance to that of II, and the ring structure of V is similar to that of III. Furthermore, the methyl group of IV and the 7-methyl group of II occupy the same position when one structure is superimposed on the other. In a similar manner, it can be readily visualized that the methyl group of V matches the position of the methyl group of the carcinogenic III*b*.

Some carcinogenic agents also inhibit tumor growth (28, 34). (The antitumor activity of the potent carcinogen II*c*, NSC-408823, is illustrated in Table I.) Conversely, many antineoplastic agents were reported to be carcinogenic (35, 36). The remarkable structural similarity between the aforementioned antileukemic alkaloids and the carcinogenic methylated polycyclic hydrocarbons naturally led to a concern that these alkaloids and related compounds may possess the undesired oncogenic action.

A comparison of the structures of IV and V with those of the methylated benzanthracenes II*a*–II*c* and chrysenes III*a*–III*c* revealed that, although both alkaloids contain the methyl groups at positions corresponding to the polycyclic hydrocarbon sites that manifest carcinogenicity, the presence of the quaternized nitrogen atom of coralyne and nitidine close to the K and L regions of the corresponding polynuclear ring systems may drastically modify the chemical reactivity of the original molecule, which may alleviate the biological action toward carcinogenesis. In addition, the alkoxy groups of both alkaloids are situated at the corresponding M region, which may also interfere with the oncogenic *in vivo* epoxidation–perhydroxylation process.

Introduction of nitrogen atoms into carcinogenic polycyclic ring compounds, depending upon the nature of ring systems and the position of nitrogen atoms, may either produce compounds with increased carcinogenicity (37–42), decreased carcinogenicity (43), similar biological activity (44), or carcinolytic activity (45). There is, as yet, little information concerning the relationship between quaternized nitrogen het-

Table I—Antitumor Activity of 7,12-Dimethylbenz[*a*]-anthracene[a]

Test System	Dose[b], mg/kg	Survivors[c]	Animal Weight Difference, T − C[d]	T/C[e], %
Ca-755	38	58/60	−0.2	32
	19	18/20	0.2	32
	9	9/10	0.3	44
S-180	94	18/24	−1.1	37
	71	4/6	−0.9	69
	50	6/6	−0.2	37
L-1210	200	6/6	−5.2	165
	100	6/6	−4.2	143
	50	6/6	−4.6	147
	37.5	6/6	−1.4	145
	25	12/12	−1.8	139
	16.5	6/6	−1.7	141
	12.5	6/6	−1.2	126
	6.25	6/6	0.1	123
P-388	13	6/6	−2.4	172
	12.5	6/6	−3.0	175
	6.25	6/6	−2.2	180
	6	6/6	−1.5	168
	4	12/12	−2.7	170
	2.6	6/6	−1.7	163
	1.7	6/6	−2.0	172

[a] For general screening procedure and data interpretation, see R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972), and Instruction Booklet 14, "Screening Data Summary Interpretation and Outline of Current Screen," Drug Evaluation Branch, Drug Research and Development, Division of Drug Treatment, National Cancer Institute, Bethesda, Md., 1974. [b] Single dose daily. [c] Ratio of number of animals alive on day of evaluation to number of animals used at the beginning of the evaluation. [d] Average weight change of test group minus average weight change of control animals in grams measured at the end of the evaluation. [e] Ratio of test evaluation to control evaluation. For Ca-755 and S-180 (tumor inhibition systems), the criterion for minimum activity is T/C ≤ 42%. For L-1210 and P-388 (survival tumor systems), the criterion for minimum activity is T/C ≥ 125%.

erocycles and carcinogenicity. As an initial step to test the postulation that quaternized nitrogen atoms close to the known "oncogenic area" may alleviate carcinogenicity, coralyne sulfoacetate (IV, X = $C_2H_3SO_5$, NSC-154890), nitidine chloride (V, X = Cl, NSC-146397), nitidine methosulfate (V, X = CH_3SO_4, NSC-160842), the 8-ethyl homolog of coralyne (VI, homocoralyne, NSC-156625), and the tetramethoxy analog of nitidine (VII, *O*-methylfagaronine, NSC-166720), together with IIc as a standard, were subjected to the Ames method for mutagenicity evaluation (46–49). The relationship of mutagenicity and carcinogenicity was discussed previously (46–55).

Mutagenicity tests were carried out according to the specific procedure developed by Ames *et al.* (47) using the histidine–auxotroph strains of *Salmonella typhimurium* TA-1537, TA-1538, TA-98, and TA-100. In this system, petri dishes containing minimal agar were overlayed with a soft agar containing 9000×*g* liver supernate from phenobarbital-induced rats, an NADPH-generating system, bacteria, and various concentrations of test chemical or reference standard. The cultures were incubated at 37° for 72 hr, after which the number of histidine-revertant colonies per plate was determined.

Table II shows the mutagenic effect of IIc and the five test compounds on the *Salmonella* indicator organisms. Compound IIc was mutagenic in all strains. The mutagenic response was high for IIc in TA-1537, TA-1538, TA-98, and TA-100, averaging a three- to 10-fold increase in the number of revertants. With the exception of TA-100, a very sensitive strain, the mutagenic response was absent with all five coralyne and nitidine compounds tested. Even with TA-100, *O*-methylfagaronine methosulfate and coralyne sulfoacetate were nonmutagenic. The dose-response curve for Strain TA-100 is shown in Fig. 1. Similar curves were obtained for all positive strains.

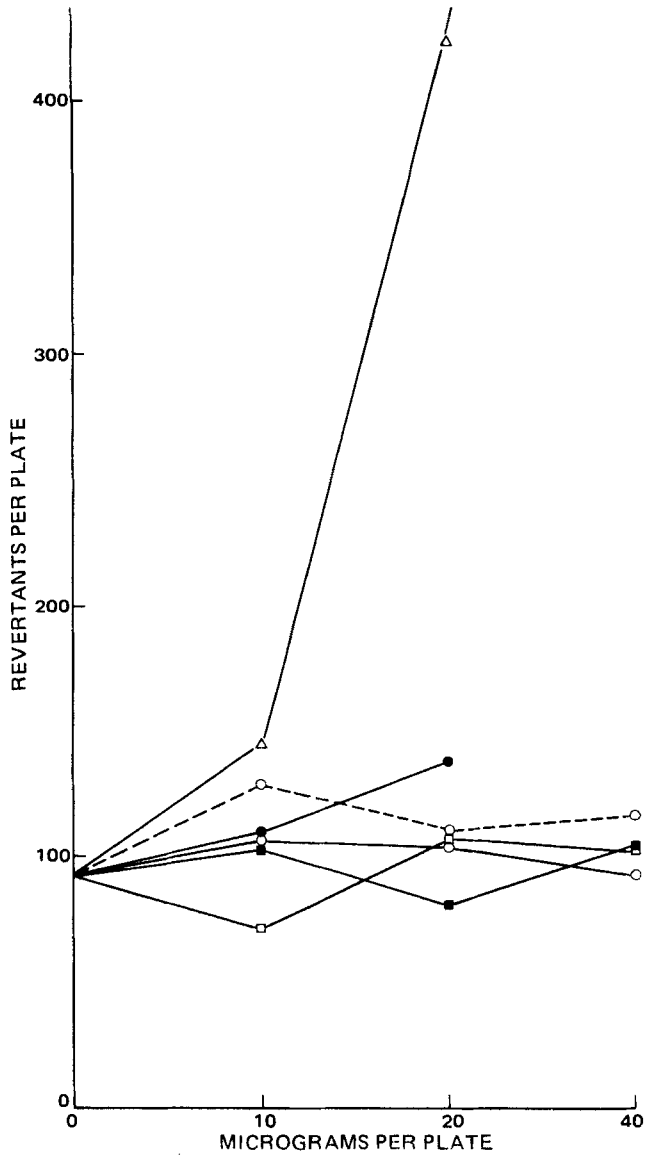


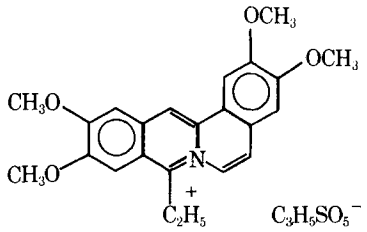
Figure 1—*Mutagenic dose–response relationship of IIc, coralyne, nitidine, and related compounds* versus S. typhimurium *TA-100. Key:* △, *IIc;* ●, *nitidine chloride;* - - - ○ - - -, *nitidine methosulfate;* —○—, *coralyne sulfoacetate;* ■, *O-methylfagaronine; and* □, *homocoralyne sulfopropionate.*

These results indicate that the presence of a quaternary nitrogen atom and alkoxy groups at strategic positions may be important in alleviating mutagenicity of the parent mutagenic and carcinogenic hydrocarbons.

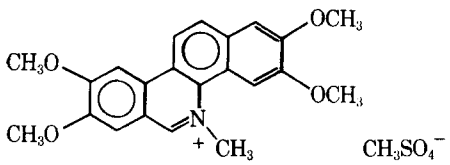
Table II—Mutagenicity Evaluation of 7,12-Dimethylbenz[*a*]anthracene, Coralyne, Nitidine, and Related Compounds

Compounds Tested (40 μg/plate)	Number of Histidine Revertants (Histidine Revertants per Nanomole)[a]			
	TA-1537	TA-1538	TA-98	TA-100
IIc	30 (0.18[c])	22 (0.10[c])	94 (0.47[c])	425 (2.13[c])
Nitidine chloride[b]	5 (0.03)	10 (0.04)	30 (0.10)	117 (0.25[c])
O-Methylfagaronine	6 (0.04)	4 (0)	25 (0.06)	95 (0.02)
Homocoralyne sulfopropionate	2 (0)	4 (0)	20 (0)	103 (0.14[c,d])
Coralyne sulfoacetate	4 (0.02)	8 (0.02)	20 (0)	94 (0.01)
Nitidine methosulfate	3 (0.01)	6 (0)	22 (0.02)	117 (0.29[c])
Control	2	6	20	93

[a] Number of revertants (R) per nanomole = $(R_t - R_c)/(C/mw)$, where R_t is the number of revertants in the treated dish, R_c is the number of revertants in the control dish, C is the concentration of test compound in nanograms, and mw is the molecular weight of the test compound. [b] Due to the insolubility of this compound, only 20 μg was used. [c] Classified to be mutagenic by Ames' criteria (48), *i.e.*, > 0.1 revertant/nmole. [d] Not significantly different from the control value. The reproducibility of the test is ±15%.



VI



VII

While the relationship between mutagenicity and carcinogenicity of biologically active compounds cannot be fully correlated, its closeness has been recognized. Therefore, the results can be regarded as *suggestive* of the absence of carcinogenicity of coralyne and related antileukemic agents.

REFERENCES

(1) C. E. Dunlap and S. Warren, *Cancer Res.*, **3,** 606 (1943).
(2) A. Pullman and B. Pullman, *Adv. Cancer Res.*, **3,** 117 (1955).
(3) W. F. Dunning and M. R. Curtis, *J. Natl. Cancer Inst.*, **25,** 387 (1960).
(4) A. Lacassagne, F. Zajdela, N. P. Buu-Hoï, and O. Chalvet, *Bull. Cancer*, **49,** 312 (1962).
(5) D. Hoffmann, W. E. Bondinell, and E. L. Wynder, *Science*, **183,** 215 (1974).
(6) M. M. Coombs, T. S. Bhatt, M. Hall, and C. J. Croft, *Cancer Res.*, **34,** 1315 (1974).
(7) F. Bergmann, *ibid.*, **2,** 660 (1942).
(8) A. Pullman and B. Pullman, *Acta Unio. Int. Cancrum*, **6,** 57 (1948).
(9) J. E. Lovelock, A. Zlatkis, and R. S. Becker, *Nature*, **193,** 540 (1962).
(10) Y. Valladares, *Acta Oncol.*, **8,** 3 (1969).
(11) A. Pullman and B. Pullman, *Nature*, **196,** 228 (1962).
(12) J. A. Miller and E. C. Miller, *Cancer Res.*, **23,** 229 (1963).
(13) W. Lijinsky, H. Garcia, and U. Saffiotti, *J. Natl. Cancer Inst.*, **44,** 641 (1970).
(14) J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia*, **28,** 1129 (1972).
(15) N. Kinoshita, B. Shears, and H. V. Gelboin, *Cancer Res.*, **33,** 1937
(16) P. B. Hulbert, *Nature*, **256,** 146 (1975).
(17) P. L. Grover, P. Sims, B. C. V. Mitchley, and F. J. C. Roe, *Br. J. Cancer*, **31,** 182 (1975).
(18) W. C. Herndon, *Trans. N.Y. Acad. Sci.* [*II*], **36,** 200 (1974).
(19) D. M. Jerina and J. W. Daly, *Science*, **185,** 573 (1974).
(20) A. Flaks and P. Sims, *Br. J. Cancer*, **32,** 604 (1975).
(21) P. Y. Bruice, T. C. Bruice, P. M. Dansette, H. G. Selander, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **98,** 2965 (1976).
(22) H. Marquardt, P. L. Grover, and P. Sims, *Cancer Res.*, **36,** 2059 (1976).
(23) F. A. Beland and R. G. Harvey, *J. Am. Chem. Soc.*, **98,** 4963 (1976).
(24) K. Y. Zee-Cheng and C. C. Cheng, *J. Pharm. Sci.*, **61,** 969 (1972).
(25) K. Y. Zee-Cheng, K. D. Paull, and C. C. Cheng, *J. Med. Chem.*, **17,** 347 (1974).
(26) R. K. Y. Zee-Cheng and C. C. Cheng, *ibid.*, **18,** 66 (1975).
(27) *Ibid.*, **19,** 882 (1976).
(28) A. Haddow and A. M. Robinson, *Proc. Roy. Soc. B*, **122,** 442 (1937).
(29) A. Haddow, *J. Pathol. Bacteriol.*, **47,** 567, 581 (1938).
(30) G. M. Badger and J. W. Cook, *J. Chem. Soc.*, **1940,** 409.
(31) T. B. Sorokina, *Vopr. Khim. Zlok. Opukh.*, **11,** 152 (1960); through *Cancer Chemother. Abstr.*, **3,** 62 (1962).
(32) V. Armaghan, *J. Natl. Cancer Inst.*, **25,** 125 (1960).
(33) V. Armaghan, *Cancer Chemother. Rep.*, **14,** 117 (1961).
(34) N. P. Buu-Hoï, *Med. Exp.*, **8,** 209 (1963).
(35) J. A. Miller, *Cancer Res.*, **30,** 559 (1970).
(36) C. C. Harris, *Cancer*, **37,** 1014 (1976), and references cited therein.
(37) E. Boyland and A. M. Brues, *Proc. Roy. Soc. B*, **122,** 429 (1937).
(38) E. Boyland and E. H. Manson, *Biochem. J.*, **32,** 1640 (1938).
(39) C. Hackmann, *Z. Krebforsch.*, **58,** 56 (1951).
(40) A. Lacassagne, N. P. Buu-Hoï, F. Zajdela, and N. D. Xuong, *Bull. Cancer*, **42,** 3 (1955).
(41) A. Lacassagne, N. P. Buu-Hoï, R. Daudel, and F. Zajdela, *Adv. Cancer Res.*, **4,** 315 (1956).
(42) A. Lacassagne, N. P. Buu-Hoï, F. Zajdela, F. Périn, and P. Jacquignon, *Nature*, **191,** 1005 (1961).
(43) M. J. Shear and J. Leiter, *J. Natl. Cancer Inst.*, **2,** 99 (1941).
(44) P. Markovits, J. Coppey, D. Papadopoulo, A. Mazabraud, and M. Hubert-Habart, *Int. J. Cancer*, **14,** 215 (1974).
(45) J. Moszew and B. Adamczyk, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.*, **13,** 383 (1965).
(46) B. N. Ames, in "Chemical Mutagens," vol. 1, H. Hollander, Ed., Plenum, New York, N.Y., 1971, p. 267.
(47) B. N. Ames, J. McCann, and E. Yamasaki, *Mutation Res.*, **31,** 347 (1975).
(48) J. McCann, E. Choi, E. Yamasaki, and B. N. Ames, *Proc. Natl. Acad. Sci. USA*, **72,** 5135 (1975).
(49) J. McCann and B. N. Ames, *ibid.*, **73,** 950 (1976).
(50) G. Obe, K. Sperling, and H. J. Belitz, *Angew. Chem.*, **83,** 301 (1971).
(51) J. P. Seiler, *Chimia*, **29,** 8 (1975).
(52) E. Huberman and L. Sachs, *Proc. Natl. Acad. Sci. USA*, **73,** 188 (1976).
(53) E. Huberman, L. Sachs, S. K. Yang, and H. V. Gelboin, *ibid.*, **73,** 607 (1976).
(54) B. A. Bridges, *Nature*, **261,** 195 (1976).
(55) J. McCann, *Chem. Technol.*, **1976,** 682.

ACKNOWLEDGMENTS AND ADDRESSES

Received December 13, 1976, from the *Midwest Research Institute, Kansas City, MO 64110, and the ‡National Cancer Institute, Bethesda, MD 20014.

Accepted for publication February 16, 1977.

Supported by Contract N01-CM-33743 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.

The authors thank Mary Kowalski and Tonya Olmstead for technical assistance in the mutagenesis testing.

ˣ To whom inquiries should be directed.