in 30 mL of MeOH was stirred overnight with 4.2 g (30 mmol) of powdered anhydrous K_2CO_3 and 2.95 mL of (4-fluorophenyl)acetyl chloride [prepared by reaction of (4-fluorophenyl)acetic acid with $SOCl_2$]. The mixture was diluted with 5 mL of H_2O and evaporated to a white slurry. An additional 20 mL of H_2O was added; the mixture was extracted (2 × 20 mL of $CHCl_3$) and the combined extract was dried (Na_2SO_4) and evaporated, yielding 3.82 g of syrup. Crystallization from ligroin/ether gave 3.06 g of white solid, which was reduced directly as follows.

A 1.00 g (2.8 mmol) portion of the solid was dissolved in 10 mL of dry THF and refluxed with 11.0 mL of 1.0 M B_2H_6 in THF. After 2 h, 5 mL of MeOH was cautiously added, and the mixture was evaporated to a syrup and then taken up in 20 mL of MeOH and stirred with 5 mL of 37% HCl at 90 °C (bath temperature) for 1 h. Evaporation of the MeOH left a residue, which was treated with 20 mL of H_2O, neutralized (NH_4OH, pH 7), and extracted (3 × 50 mL of $CHCl_3$). The $CHCl_3$ was dried (Na_2SO_4) and evaporated to give 980 mg of foam, which was dissolved in 5 mL of 2-propanol, acidified with a few drops of 48% HBr, and then diluted with 3 mL of ether to yield 847 mg of **3**·HBr as white prisms: mp 150–153 °C; CIMS (CH_4), QM 340; NMR ($CDCl_3$, free amine) δ 0.86 (d, 3 H, J = 7 Hz), 1.32 (s, 3 H), 1.43–1.25 (m, 1 H), 2.36–1.89 (m, 3 H), 3.00–2.68 (m, 6 H), 3.20 (br s, 1 H), 6.68 (dd, 1 H, J = 8 and 2 Hz), 6.76 (d, 1 H, J = 2 Hz), 7.00–6.86 (m, 3 H), 7.16–7.07 (m, 3 H); IR ($CHCl_3$, free amine) 3300, 2960–2841, 1610, 1586, 1494 cm^{-1}; NMR of the HBr salt confirmed the presence of 1 mol of 2-propanol of solvation. Anal. Calcd for $C_{22}H_{26}FNO·HBr·C_3H_8O·^1/_2H_2O$: C, 61.35; H, 7.41; N, 2.86. Found: C, 60.99; H, 7.32; N, 2.82.

Biochemical Methods. For the binding assays, male Sprague–Dawley rats were killed by decapitation, the brains were rapidly removed, and the cerebellum was dissected away. The brains were homogenized in 50 vol of 0.05 M Tris–HCl buffer, pH 7.4, at 4 °C in a Brinkman Polytron (setting 6). The resulting homogenate was centrifuged at 18000 rpm for 15 min. After centrifugation, the supernatant was decanted and the pellets of the crude membrane preparation were resuspended in 2.5 vol of 0.05 M Tris-HCl, pH 7.4, and frozen. In the standard binding assay, 1-mL aliquots of the thawed crude membrane preparation were incubated in triplicate. For [^{3}H]DADLE, the incubation medium consisted of 0.05 M Tris-HCl at pH 7.4 and 1% bovine serum albumin. For naloxone, the medium consisted of 0.05 M Tris-HCl, 100 mM NaCl, 2 μM GTP, bacitracin at 0.5 mg mL^{-1}, and aprotinin at 100 kallikrein inhibitory units/mL. For EKC, the medium consisted of 0.05 M K_2HPO_4·HCl buffer, 1 mM EDTA, 100 mM NaCl, pH 7.4. To each was added the appropriate ^{3}H-labeled ligand at about 50000 cpm with either unlabeled ligand, unlabeled phenazocine, or unlabeled fluorophen to give a final dilution of 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} M. The resulting mixtures were incubated at 25 ([^{3}H]DADLE) or 4 °C ([^{3}H]naloxone and [^{3}H]-EKC) for 30 (if 25 °C) or 60 min (if 4 °C), and incubation was terminated by rapid filtration through glass-fiber circles (Whatman GF/B) and rinsed twice with cold buffer. Binding of ^{3}H-labeled ligand was quantitated by measuring the radioactivity of the membrane-laden filter in 10 mL of scintillation cocktail after agitation of the counting vials for 30 min.

Bioassays were prepared as follows. For guinea pig ileum, male guinea pigs weighing 400 to 450 g were sacrificed by decapitation, and the terminal ileum was exposed and dissected free. The distal 10 cm of ileum adjacent to the ileocecal junction was discarded. A portion of ileum approximately 10 cm in length was slid onto a glass rod, and the longitudinal muscle was mobilized from the circular muscle along the mesenteric border by careful stroking with a cotton pledget soaked in Krebs solution. The entire longitudinal muscle with the adherent myenteric plexus was then separated from the circular muscle by gentle blunt dissection. For mouse vas deferens, male mice weighing 20 to 25 g were killed by decapitation, and the vasa deferentia were dissected out en bloc with the seminal vesicle. Adherent blood vessels and connective tissue were removed by careful blunt dissection, the vasa were separated from the seminal vesicle, and the contents were expelled. For rabbit vas deferens, male rabbits weighing between 2500 and 3000 g were killed and decapitated, and the vasa deferentia were prepared as above. After dissection, all tissues were placed in organ baths in warm Krebs solution (NaCl, 118 mM; KCl, 4.75 mM; $CaCl_2$, 2.54 mM; K_2HPO_4, 1.19 mM; $NaHCO_3$, 25 mM; and glucose, 11 mM, at 37 °C), gassed with 95% O_2/5% CO_2, and placed under resting tension (1000 mg for guinea pig ileum, 100 mg for mouse vas deferens, and 500 mg for rabbit vas deferens). Platinum electrodes were used to deliver stimuli (supramaximal rectilinear pulses of 0.1-ms duration at 0.1 Hz) to the myenteric plexus or the intramural nerves. Contractions were recorded isometrically (Grass transducers, FT 03) and displayed on a Grass polygraph.

Registry No. (±)-1, 52079-30-8; (±)-2, 86436-93-3; (±)-3, 86495-14-9; (±)-3·HBr, 86436-94-4; (*p*-fluorophenyl)acetyl chloride, 459-04-1.

(22) Fry, E. M.; May, E. L. *J. Org. Chem.*, **1959**, *24*, 116.

Some Biological Properties of the Impure Dichloride Salt of Tetrakis[*p*-(dimethylamino)phenyl]ethylene and a Pinacolone

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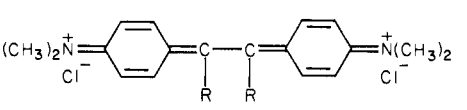
The impure dichloride salt of tetrakis[*p*-(dimethylamino)phenyl]ethylene and a pinacolone that is a substituted acetophenone show several biological properties, one of which is activity against lymphosarcoma in mice. The involvement, if any, of free radicals in the biological properties of these substances is discussed.

Work[1] carried out in our laboratories has shown that the unpurified salt (1)[2] of tetrakis[*p*-(dimethylamino)-phenyl]ethylene has some biological activity. Subcutaneous injection of impure 1 into rats depressed the leucocyte count and prevented tumor formation in rats fed



1, R = $(CH_3)_2NC_6H_4$-

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(dimethylamino)azobenzene. These results were not confirmed when pure **1**, which had been made from the dinitrate salt and sodium chloride, was used.

We now report work carried out in our laboratories, which indicates that impure **1** contains some of the HCl salt of tetrakis[*p*-(dimethylamino)phenyl]ethylene (**2**).

$$[(CH_3)_2NC_6H_4]_2C{=}C[C_6H_4N(CH_3)_2]_2$$

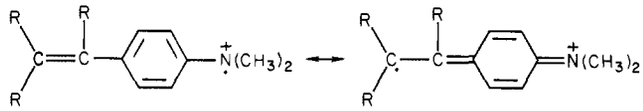
2

This salt may be formed whenever **1** is made by the addition of chlorine to a solution of **2** in CCl_4. Both salts may also be produced when **2** in CCl_4 is irradiated with light from a Hanovia ultraviolet quartz lamp. Heating of **1** in air at 140 °C for a short period of time may afford a mixture of **1** and **2**·HCl.

Separation of the mixture is carried out by extracting with $CHCl_3$, in which **1** is soluble. The HCl salt is not soluble and is removed by filtration. Addition of CCl_4 to the $CHCl_3$ filtrate affords **1**.

We have found that a mixture with a Cl^- content of 13.82% and made by adding chlorine as phenyliodoso chloride to **2** in $CHCl_3$ is active against lymphosarcoma in mice. In contrast to this, **1** by itself and the HCl salt of **2** by itself have no effect (Table I). Moreover, the IR spectrum of the mixture that is active against lymphosarcoma in mice is similar to the IR spectrum of a mixture made from the two separately and previously prepared components. The spectra of the biologically active and prepared mixtures show weak benzenoid absorption at 1510 cm^{-1} and intense quinoid absorption at 1580 cm^{-1}. The spectrum of the HCl salt of **2** shows strong absorption at 2250 cm^{-1}, intense benzenoid absorption at 1510 cm^{-1}, and weak absorption at 1600 cm^{-1}. The latter absorption may be due to some dichloride salt that is produced slowly in air. The absorption at 2250 cm^{-1} is weak or absent in the spectra of the mixtures.

The spectral data suggest that the mixtures may be compounds similar to quinhydrone in which hydrogen bonding is involved. In the case of the active and prepared mixtures, the reductant is the HCl salt of **2** and the oxidant is the dichloride salt of **2**. The hydrogen of the HCl salt affords the hydrogen bonding. Quinhydrone gives the semiquinone free radical. In a like manner, the active and prepared mixtures give the radical ion. **3**.



3 **3**

etc.

Electron-spin resonance (ESR) spectra of mixtures of **1** and the HCl salt of **2** in the solid state[4] or in water solution show a free-radical content. Also, mixtures of **1** and **2** in the solid state[4] or in ethylene chloride solution show intense ESR signals. This property is absent or much diminished when the two components are studied separately. We consider that the formation of the radical ion **3** from the active and prepared mixtures is possible and may be accounted for by disproportionation according to eq 1.

olefin (**2**) (reductant) + dichloride salt (**1**) (oxidant) → 2 radical ion (**3**) + $2Cl^-$ (1)

The biologically active mixture also prevents the formation of a gel in the system benzene, olefin (**2**), and acetic acid.[9] A solution of olefin (**2**) in benzene and acetic acid in air becomes dark in color and, finally, becomes a gel. It involves a free-radical reaction and is prevented by antioxidants such as hydroquinone and ascorbic acid. The dichloride salt (**1**), one of the components of the biologically active mixture, does not prevent gelation, and this is to be expected. However, the HCl salt of **2**, which is the other component of the active mixture, prevents gelation but only because the strong acid favors the slow two-electron oxidation to the dication, which appears as the black, loose, flocculent dichloride salt. The formation of a gel from **2** requires a one-electron oxidation, and this takes place in very dilute acetic acid.

Work[1] carried out in our laboratories has shown that 4′-(dimethylamino)-2,2,2-tris[*p*-(dimethylamino)phenyl]-acetophenone (**4**),[6] which undergoes homolytic cleavage to

$$(CH_3)_2NC_6H_4{-}\overset{\overset{O}{\|}}{C}{-}C[C_6H_4N(CH_3)_2]_3$$

4

afford two free radicals[7] and given as a dilute aqueous HCl solution[8] by using the infusion technique, is active against the Walker 256 tumor in rats. It also lowers the leucocyte count in rats. We have also found that pinacolone (**4**) in $(CH_3)_2SO$ is active against lymphosarcoma in mice (Table I), and this solution also lowers the leucocyte count in rats.

Discussion

It is only on the basis of spectral data that the authors of this paper conclude that the mixture that is biologically active is the same as the mixture made from the dichloride salt (**1**) and the olefin hydrochloride salt. Due to the departure of one of us (G.A.L.), it has been impossible to carry out biological assays on prepared mixtures. For that reason the identity problem remains unresolved, and the presence of a trace contaminant of high potency is not ruled out. This difficulty is not present in the case of pinacolone (**4**), which is a pure compound and not a mixture and shows biological activity.

What involvement, if any, have radicals and radical ions in the "anticancer activity"[5] of some substances? The present work points to the radical ion (**3**) and not the olefin (**2**) or its HCl salt (reducant) and not the dichloride salt (**1**) (oxidant), which is necessary for activity against lymphosarcoma in mice (Table I). The activity of pinacolone (**4**) may be due to free radicals. Ascorbic acid, which is an antioxidant and prevents the gelation of olefin (**2**), benzene, and acetic acid, also affords a free radical. The

(2) Wizinger, R.; Fontaine, J. *Ber Dtsch. Chem. Ges.* **1927**, *60*, 1377.
(3) Lucas, R. J.; Kennedy, E. R. *Org. Synth.* **1942**, *22*, 69.
(4) The solid mixture is made by careful griding in a mortar with pestle.
(5) For the meaning and evaluation of the term "anticancer activity", see Schabel, F. M., Jr. "Nucleoside Analogues"; Walker, R. T.; DeCleroq, E.; Eckstein, F., Eds.; Plenum Press: New York, 1979.
(6) Elofson, R. M. *J. Org. Chem.* **1966**, *25*, 305.
(7) Sandin, R. B.; Elofson, R. M.; Schulz, K. F. *Can. J. Chem.* **1967**, *45*, 203.
(8) Pinacolone (**4**) trihydrochloride, (Cl^-, 16.69) is pale green in H_2O solution. It becomes more intensely green if the temperature is raised and returns to the original color if the temperature is then lowered. Evaporation of the HCl solution of pinacolone (**4**) at room temperature in vacuo affords the HCl salt.
(9) Elofson, R. M.; Schulz, K. F.; Sandin, R. B. *Can. J. Chem.* **1969**, *47*, 4447. A solution of olefin (**2**) (0.5 g) in benzene (25 mL) and acetic acid (2 drops) is allowed to stand in air, at room temperature, and in the absence of light for 48 h. The amount of test substance to be used is 10 mg.

Table I

compd no. and mixture no.	dose, mg/kg, in mouse	antilymphosarcoma act.: av survival, days	no. of mice with 50-day survival: time/no. of mice treated	control: av survival, days	control: no. of mice with 50-day survival time/no. of mice treated
I[a]	10	42.8 ± 11.5	4/5	23 ± 11.2	1/5[f]
II[b]	5	21.5 ± 6.3	1/5	14.8 ± 0.8	0/5
II[b]	10	37.6 ± 1	4/5	14.8 ± 0.8	0/5
III[c]	10	13.2 ± 1	0/5	15.6 ± 3.5	0/5
IV[d]	10	10.6 ± 0.5	0/5	12.2 ± 0.9	0/5
V[e]	5	22.6 ± 0.5	1/5	15 ± 2.8	0/5
VI[e]	10	25 ± 10.2	1/5	15 ± 2.8	0/5

[a] Mixture of 1 and 2·HCl made by adding phenyliodoso chloride in $CHCl_3$ to 2 in $CHCl_3$; Cl^-, 13.82. [b] Mixture of 1 and 2·HCl made by heating 1 at 140 °C for 5 min, extracting with hot H_2O, filtering, and evaporating the filtrate to dryness to afford black scales; Cl^-, 17.94. This procedure is drastic and the conditions are difficult to control. [c] Dichloride salt of 2. [d] HCl salt of 2; Cl^-, 20.50. [e] Pinacolone (4) in $(CH_3)_2SO$. [f] The long-term survivor had a solid tumor, indicating that cells had accidentally been given subcutaneously. Such solid tumors kill the mouse only after a long period of time. The long-term survivors in the treated groups appeared tumor free at 50 days. Note: Dose levels used did not cause weight loss. Doubled doses were not more effective where activity was obtained.

formation and properties of the ascorbic acid radical are well documented. Does this radical account for some of the biological activity[10] of ascorbic acid?

The questions asked above remain unanswered. The departure of one of us (G.A.L.) has delayed further work from being carried out.

Experimental Section

Melting points are uncorrected and were determined in open capillaries by using a Friedrichs melting point tube. The IR spectra were recorded with a Nicolet 7199 FT-IR spectrophotometer. The ESR spectral data were taken with a Bruker ER 420 console instrument equipped with a Varian V3600 12-in. magnet and field dial controller. All compounds were known, and chloride ion analyses were carried out only as a check on purity and composition of 1 and 2·HCl and mixtures of 1 and 2·HCl.

Biological assays were carried out with groups of five C3H female mice (R.B. Jackson, Laboratory, Bar Harbor, ME) injected intraperitoneally with 7.5×10^6 cells per mouse of the Gardner lymphosarcoma (6C3 HED). Beginning 1 day later, treated mice were injected subcutaneously on the lower back with the indicated compounds or the mixture dissolved in saline or 10% $(CH_3)_2SO$. Control mice were injected with the vehicle. Injections were once daily for 5 days. The data from the antilymphosarcoma testing are shown in Table I. Similar experiments with mice bearing the L1210 and P388 leukemias using mixture II[b] (Table I) were uniformly negative.

Tetrakis[*p*-(dimethylamino)phenyl]ethylene (2). The reductive coupling of Michler's ketone was carried out according to the procedure of Willstatter and Goldman.[11] The following is a modified procedure using larger quantities of starting materials. Michler's ketone (Eastman 243; 50 g), tin (90 g), and concentrated HCl (750 mL) was shaken to give a yellow solution. After standing overnight, the mixture was boiled for 2 h. Boiling H_2O (1 L) was added, and the mixture was heated until all went into solution, except some tin. The clear solution was cooled in an ice bath, and the crystalline product obtained was dissolved in warm H_2O. The solution was filtered if necessary and made alkaline with dilute NaOH. The yellow olefin (2) was filtered and washed with H_2O until free from NaOH. The olefin was air-dried, dissolved in boiling C_6H_6 (150 mL), and filtered from tin. Rapid cooling and stirring of the solution afforded the yellow olefin: yield 27.5 g (61%); mp 305–310 °C (lit.[11] mp 295 °C).

Tetrakis[*p*-(dimethylamino)phenyl]ethylene Dichloride (1). The procedure of Wizinger and Fontaine[2] for the preparation of 1 involves the addition of chlorine to a solution of 2 in CCl_4. The salt (1) is also produced when 2 dissolved in CCl_4 is irradiated with light from a Hanovia ultraviolet quartz lamp.

In the present work, 1 has been made by the dropwise addition, with stirring, of an ice-cold solution of freshly prepared phenyliodoso chloride[3] (1.2 g in 50 mL of $CHCl_3$) to an ice-cold solution of 2 (2 g) in $CHCl_3$ (50 mL). The mixture was allowed to stand at room temperature for 36 h, after which CCl_4 (400 mL) was added with stirring, and allowed to stand at room temperature for 48 h. The solid was filtered, washed with CCl_4, and dried in vacuo to afford 1 (2.4 g). Anal. Calcd for $C_{34}H_{40}Cl_2N_4$: Cl, 12.34. Found: Cl, 12.24 and 12.47.

The dichloride salt (1) has also been made from the dinitrate salt in H_2O and NaCl in H_2O. The solid dichloride salt was filtered, dissolved in H_2O, and again precipitated with NaCl in H_2O. The solid was filtered, crystallized from H_2O, air-dried, and in order to remove NaCl, dissolved in $CHCl_3$, filtered and precipitated with CCl_4, filtered, and air-dried to afford $1.8H_2O$. Anal. Calcd for $C_{34}H_{40}Cl_2N_4 \cdot 8H_2O$: Cl, 9.93. Found: Cl, 9.83 and 9.67. Attempts to make the anhydrous salt from this compound have not been successful.

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Registry No. 1, 86669-21-8; 2·HCl, 86669-22-9; 4, 14500-16-4; 4·3HCl, 86669-23-0; phenyliodoso chloride, 932-72-9; Michler's ketone, 90-94-8.

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(11) Willstatter, R.; Goldman, M. *Ber Dtsch. Chem. Ges.* **1906**, *39*, 1775.