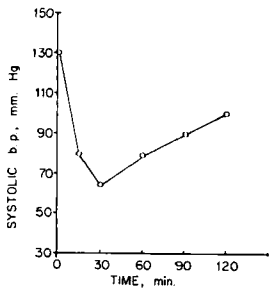

Fig. 8—The effect of Compound 1 on systolic blood pressure, 4 mg./kg. i.p.

RESULTS

The oral administration of 4 mg./kg. of Compound 1, *N*-(*p*-methoxybenzoyl)-*N*-(2-cyanoethyl)cyclohexylamine, in propylene glycol to mice resulted in a significant reduction in the spontaneous motor activity of mice (Fig. 1). A quieting or "taming" effect was seen in the animal at this dosage. Ataxia was clearly discernable in the animal at elevated dosages, *i.e.*, 10 mg./kg. The rat responded even more dramatically to elevated dosages, assuming a cataleptoid posture.

At slightly lower oral dosages, *i.e.*, 2 mg./kg. in propylene glycol, Compounds 1 and 2, *N*-(*m*-methoxybenzyl)-*N*-(2-cyanoethyl)cyclohexylamine, produced excitation in mice, while Compound 3, the *o*-methoxybenzoyl analog caused a slight reduction in total activity (Fig. 2). This dosage level, in the case of Compound 1, rather effectively antagonized the tranquilizing activity of orally, simultaneously administered 0.4 mg./kg. doses of perphenazine (Fig. 3).

Oral doses, 2 mg./kg. in propylene glycol, of Compound 4, *N*-(*p*-chlorobenzoyl)-*N*-(2-cyanoethyl)-cyclohexylamine, produced a high degree of excitation (Fig. 4), but its antagonism to 0.4-mg./kg. doses of perphenazine was insignificant.

Although depression was the predominant symptom of animals receiving 4 mg./kg. of this compound, the degree of reduction in spontaneous motor activity was much less than in the case of Compound 1.

The *m*-chlorobenzoyl analog, Compound 5, caused excitation during the first hour of measurement, but spontaneous activity rapidly decreased during the next 1.5-hr. period. Like Compound 3, the *o*-chlorobenzoyl analog (Compound 6) produced a slight reduction in total activity.

Blood pressure is markedly depressed by Compound 1, and to a somewhat lesser degree by Compounds 2 and 4 by doses of 4 mg./kg. i.v. (Figs. 5–7).

Compound 1 produced a significant hypotensive effect when administered intraperitoneally to unanesthetized normotensive rats (Fig. 8).

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Keyphrases

N-(2-Cyanoethyl)cyclohexylamine, *N*-acyl analogs—synthesis
CNS activity—*N*-(2-cyanoethyl) cyclohexylamine *N*-acyl analogs
UV spectrophotometry—structure
IR spectrophotometry—structure

Antitumor Activity of *Juglans nigra* (Black Walnut) Extractives

By UMESH C. BHARGAVA and BERTIS A. WESTFALL

Antitumor activity of compounds present in *Juglans nigra* were studied on spontaneous and/or transplanted tumors in mice. Ellagic acid, juglone, and isolated fractions (strong acids, weak acids, and alkaloids) were injected intraperitoneally for 9–12 days. The results showed that ellagic acid, juglone, and the "strong acids" fraction depressed the tumor growth rate significantly.

CAIN (1) observed that certain water-soluble polyphenolic compounds have the property to retard the growth rate of some experimental rodent tumors; however, no proof was shown experimentally. Furthermore, certain quinone derivatives (2) have been observed to increase the life span and decrease the tumor size in mice. Since some species of walnut (3–5) contain polyphenolic and/or quinone constituents, and also since *Juglans nigra* has been reported to inhibit the growth rate of some other plants (6), it was thought that active principles of walnut

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might inhibit the tumor growth rate in animals (7). This led to the present investigation on the effect of *Juglans nigra* fractions on mammary tumors in mice. Ellagic acid (3) and juglone (8) have been found in *Juglans nigra* and were used in this study. Other pharmacologically active compounds were separated into groups, namely, strong acids, weak acids, and alkaloids, and their pharmacological actions studied. These groups were defined according to their solubility and precipitation tests. Weak acids were soluble in sodium hydroxide solution, but not in sodium bicarbonate solution at pH 7.5. This fraction probably also contained phenolic materials since they exhibit the same properties. Strong acids were soluble in sodium bicarbonate solution at pH 7.5. Other crude extractives which were soluble in acidic solution and gave a precipitation test with reagents such as Wagner's, Meyer's, or Valser's reagents were probably alkaloids. The strong acids and weak acids were extracted from the hulls and the alkaloids from the leaves.

The selection of the type of tumor for the evaluation of antitumor activity is intriguing since many compounds have been reported to inhibit the growth rate of transplanted tumors, but not the spontaneous tumors (9). Therefore, both types of tumors were used.

METHODS

Extraction of Strong Acids and Weak Acids from Hulls—Two kilograms of coarsely ground dried hulls of *Juglans nigra* which were previously macerated with 5% acetic acid was extracted with diethyl ether–butanol mixture (4:1) in a Soxhlet-type apparatus for 48 hr. and the residue obtained after evaporation of the solvent was triturated with 5% acetic acid. The dissolved portion of the residue was filtered and extracted with diethyl ether–butanol mixture (4:1). The aqueous phase was discarded. The above organic solvent layer was washed several times with $NaHCO_3$ solution at pH 7.5 and this was acidified and extracted with diethyl ether–butanol mixture, which on evaporation under vacuum yielded strong acids. The organic layer after extraction with $NaHCO_3$ solution was washed several times with 1 *N* NaOH solution and the alkaline solution was made acidic and extracted like the strong acids. The solvent phase on evaporation under vacuum yielded weak acids. The aqueous phases left after the extraction of strong acids or weak acids were discarded.

Extraction of Alkaloids from Leaves—Two kilograms of dried leaves from *Juglans nigra* was macerated with chloroform–butanol mixture (4:1) containing 5% ammonia solution overnight. Next morning the leaves were extracted for 48 hr. with chloroform–butanol mixture. The organic solvent after extraction was evaporated to semisolid residue under vacuum. The residue was triturated with 5% acetic acid. The acidic solution was rendered alkaline with ammonia solution and extracted with chloroform–butanol mixture. The organic solvent on evaporation under vacuum yielded alkaloids.

Spontaneous Tumor—Mice (40–60 g., Swiss Webster) having mammary adenocarcinoma tumors were purchased from the Taconic Farms and were fed Purina chow and water *ad libitum*. At least a week was allowed after arrival before the animals were used in the experiment.

A daily dose of ellagic acid (50 mg./kg. i.p., solutions pH 11) was injected for 9 days and tumor size (length, width, and height in mm. were multiplied) and mouse weight were measured at 0, 3, 6, and 9 days after the first injection of ellagic acid. Similarly, juglone, strong acids fraction, and weak acids fraction were injected (i.p.) in doses 10 mg./kg., 100 mg./kg., and 100 mg./kg., respectively. The tumor size and mouse weight were measured in the beginning of the experiment and then 3, 6, and 9 days after the first injection of each of the fractions.

Transplantable Tumor—Retired breeder mice (14–16 g., C57BL/6J), purchased from the Jackson Laboratory were used. They were fed Purina chow and water *ad libitum*. Ten days after receiving the animals, each mouse was transplanted subcutaneously with BW-10232 mammary adenocarcinoma in the ventrolateral region.

A daily dose of total alkaloids (50 mg./kg.) was injected (i.p.) and the tumor size and mouse weight were measured at the beginning of the experiment and then 2, 5, 9, and 12 days after the injection of alkaloids.

In another group of mice with transplanted tumors, 10 mg. of ellagic acid/kg. was injected (i.p.) twice a day, the tumor size and mouse weight were measured in the beginning, and then 3, 7, and 10 days after the first injection of ellagic acid.

Control mice were treated in a similar manner as experimental mice except saline was injected intraperitoneally instead of the black walnut fraction. In the case of ellagic acid experiment saline at pH 11 was used. Changes in tumor size and mouse weight were calculated and plotted against time (days). Standard error was calculated using the method of tests of difference or paired data as described by Snedecor (10). Corrected percent mortality of experimental animals was calculated by subtracting percent mortality of controls from percent mortality of experimental animals. Thus the percent of animals which died by chance without the treatment with drugs was eliminated.

RESULTS AND DISCUSSION

Ellagic Acid—The results in Fig. 1 show ellagic acid significantly decreased the rate of tumor growth and increased the weight of spontaneous tumor mice. The changes in tumor size and weight during the 9-day period in ellagic acid-treated mice were 1,457 ± 580 $mm.^3$ and 2.96 ± 1.08 g., respectively, as compared with controls 4,203 ± 1,116 $mm.^3$ and −0.036 ± 0.74 g., respectively, which were statistically significant ($p = 0.03$). There was more growth of tumor in the controls as compared to ellagic acid-treated animals when increase in tumor size per gram of increase in mouse weight was used.

The effect of ellagic acid on transplanted tumor mice was quite different as compared to spon-

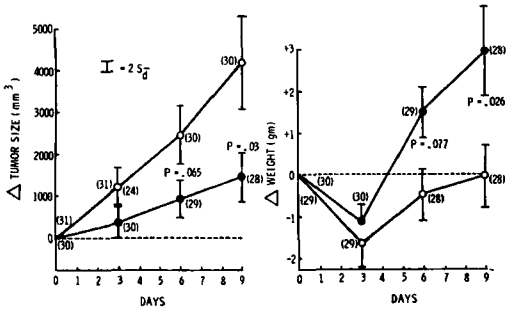

Fig. 1—Effect of ellagic acid on tumor size and body weight in spontaneous tumor mice. Change in tumor size or body weight was plotted against the days after ellagic acid treatment. Each point was obtained from the mean of the animals indicated in the bracket with the standard error. Statistical significance (p *value*) *was calculated by comparing the experimental group with the control. Key:* ○, *control;* ●, *ellagic acid.*

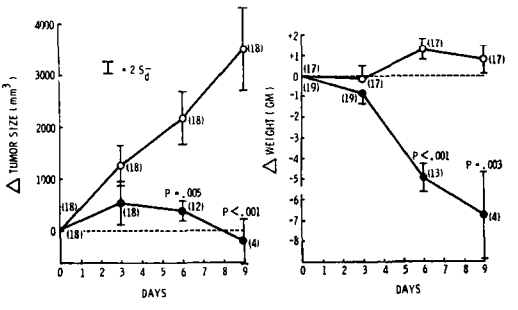

Fig. 3—Effect of juglone on tumor size and body weight in spontaneous tumor mice. Change in tumor size or body weight was plotted against the days after juglone treatment. Each point was obtained from the mean of the animals indicated in the bracket with the standard error. Statistical significance (p *value*) *was calculated by comparing the experimental group with the control. Key:* ○, *control;* ●, *juglone.*

taneous tumor mice. Figure 2 illustrates that ellagic acid had a significant effect ($p = 0.015$) on tumor growth after 6 days of treatment. But this increase in tumor size became insignificant when the mice were treated for 10 days. This might be because the rate of growth of the transplanted tumor was much faster as compared to the spontaneous tumor in respect to tumor volume and size of the animal. Unlike the spontaneous tumor mice, ellagic acid in transplanted tumor mice decreased the body weight ($p < 0.001$).

Percent mortality of mice with transplanted tumor (27.78%) receiving ellagic acid was higher as compared to spontaneous tumor mice (3.44%) receiving that compound.

Juglone—Results obtained from juglone (synthetic) treatment in spontaneous tumor mice are shown in Fig. 3. Juglone decreased significantly the gain in tumor size ($p < 0.001$) and growth in weight ($p = 0.003$) over the period of 9 days of injections. The percent mortality of juglone-treated mice (78.95%) was much higher than any other compounds used. Most of the animals developed acute diarrhea which may have contributed to their death. Therefore, in terms of drug effectiveness, it is questionable that juglone has any specific effect on tumor growth rate since the mouse body weight was also decreased significantly.

Strong Acids, Weak Acids, and Alkaloids—Figure 4 shows the effect of strong acids and weak acids on tumors. Only the strong acids group reduced (slightly) the tumor growth rate and this was statistically significant in the mice receiving this fraction for 3 days. Paper chromatography of the strong acids in isobutyl alcohol–acetic acid–distilled water (40:10:50) solvent system indicates the presence of at least three different compounds (R_f values 0.67, 0.85, and 0.95). The weak acids and alkaloids did not show any significant effect on tumor growth. In the case of alkaloids increase in tumor size and body weight were 7,837 ± 679 mm.^{3} and 3.64 ± 0.45 g., respectively, as compared to controls 7,745 ± 990 mm.^{3} and 3.76 ± 0.52 g., respectively, when the animals were treated with alkaloids for 9 days. The strong acids and weak acids did not show any significantly different effect from controls when weight changes were included in the comparison.

The percent mortality during the period of observation was higher after the animals received the strong acids (25%) than for those receiving weak acids (6.25%) or alkaloids (−5%).

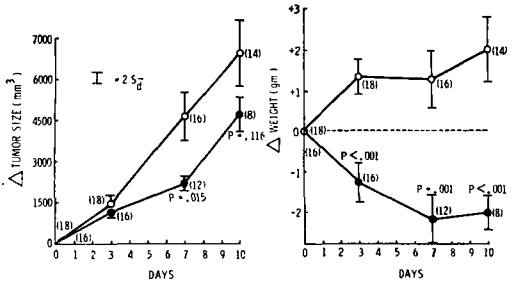

Fig. 2—Effect of ellagic acid on tumor size and body weight in transplanted tumor mice. Change in tumor size or body weight was plotted against the days after ellagic acid treatment. Each point was obtained from the mean of the animals indicated in the bracket with the standard error. Statistical significance (p *value*) *was calculated by comparing the experimental group with the control. Key:* ○, *control;* ●, *ellagic acid.*

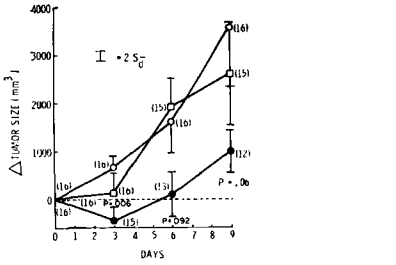

Fig. 4—Effect of strong acids or weak acids on tumor size in mice. Change in tumor size was plotted against days after strong acids or weak acids treatment. Each point was obtained from the mean of the animals indicated in the bracket with the standard error. Statistical significance (p *value*) *was calculated by comparing the experimental group with the control. Key:* ○, *control;* ●, *strong acids;* □, *weak acids.*

It would be worthwhile to isolate the pure compounds from the crude fractions; strong acids, weak acids, and alkaloids and then test them on tumor-bearing mice, because many compounds in the pure state have been reported to be highly active against certain types of leukemias, but were devoid of activity in crude fractions. For example, Svoboda (11) reported the alkaloids, leurocristine and leurosidine, were very active against P-1534 leukemia, but were relatively inactive in crude fractions from which they were isolated. A similar situation was observed by Farnsworth *et al.* (12).

SUMMARY

1. Pharmacologically active compounds present in black walnut were evaluated for antitumor activity on spontaneous and transplanted tumors in mice.

2. Ellagic acid depressed the tumor growth in both spontaneous and transplanted tumor. This effect was more apparent on the spontaneous tumors than on transplanted tumors. Ellagic acid increased the weight of mice with spontaneous tumors but decreased the weight in animals with transplanted tumors.

3. Juglone decreased the tumor growth rate and weight of the spontaneous tumor mice.

4. Strong acids slightly decreased the rate of tumor growth (spontaneous), but weak acids (spontaneous) and alkaloids (transplanted) did not have any significant effect. Furthermore, the weight of the mice was not affected by any of these three groups.

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Keyphrases

Antitumor compounds—*Juglans nigra*
Acidic components, *Juglans nigra*—antitumor activity
Ellagic acid—antitumor activity
Juglone—antitumor activity
Alkaloids, *Juglans nigra*—antitumor activity

Complex Formation Influence on Reaction Rate IV

Studies on the Kinetic Behavior of 3-Carbomethoxy-1-methylpyridinium Cation

By DANA BROOKE* and DAVID E. GUTTMAN†

The kinetics of reaction of the ester, 3-carbomethoxy-1-methylpyridinium cation (NME), were investigated in the pH region 8.0–9.8, in the absence and presence of the electron donor, 8-chlorotheophyllinate anion (CT). Hydrolysis of the ester was shown to be first order with respect to hydroxide ion in this region. The rate of hydrolysis was significantly decreased in the presence of CT. Spectral studies demonstrated the formation of a complex between the ester and CT. The rate of reaction of the complexed ester was about one-fifteenth that of the free ester. Both glycine and TRIS buffers were shown to contribute to the rate of reaction. The contribution due to glycine appeared to be the result of glycinate anion functioning as a general base catalyst. The mechanism of the TRIS reaction appeared to be different from that of glycine. The data suggested that neutral TRIS and the ester reacted by two different pathways to form an unstable ester or a stable amide.

N[1]-ALKYLPYRIDINIUM cations have been extensively used as model compounds in assessing the chemical and physical-chemical properties of pyridine coenzymes. Investigations of the kinetic and complexometric behavior of such cations are, therefore, of general interest. The present investigation was initiated to study the possible effects of complex formation upon the kinetic behavior of the ester, 3-carbomethoxy-1-

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