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PLANT ANTIMUTAGENIC AGENTS, 3.[1] COUMARINS.

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ABSTRACT.—Several coumarins were isolated from crude plant extracts by means of an antimutagenic assay procedure. These coumarins included psoralen from *Psoralea corylifolia* and imperatorin and osthol from *Selinum monniere*. Studies of structure-activity relationships of these and several other available coumarins were carried out with four mutagens. All of the coumarins were nontoxic and in particular showed high activity in the inhibition of the mutagenicity of benzo[*a*]pyrene.

Coumarins are one of the most common plant secondary metabolites, over 800 being reported in a comprehensive survey (2). Recently there has been considerable interest in the anticarcinogenic or antimutagenic activity of some of these compounds. Thus, coumarin [**1**], the simplest member of this family, has been reported to be an inhibitor of dimethylbenz[*a*]anthracene-induced mammary neoplasia, whereas umbelliferone [**2**] (7-hydroxycoumarin) was inactive (3). Ohta *et al.* (4) report that **1** and **2** showed antimutagenic action toward the mutagenic effects of 4-nitroquinoline-1-oxide and uv irradiation on *Escherichia coli* (WP2s). Recently we have shown that crude plant extracts can be rapidly and efficiently screened for possible antimutagenic activity (5) utilizing the measurement of the inhibitory action of such extracts on the mutagenicity of 2-aminoanthracene (2AN), plus the Ames S-9 enzyme preparation toward *Salmonella typhimurium* (T-98) (6,7), and we have examined the effects of flavonoids in this assay (1). In this paper we wish to present the utilization of the above procedure for the isolation of certain coumarins from wild plants and also to present certain structure-activity relationships involving the interaction of a number of coumarins with several mutagens.

EXPERIMENTAL

MUTAGENIC INHIBITION.—Inhibition of the mutagenic activity of 2-aminoanthracene (2AN) toward *S. typhimurium* (T-98) by the various coumarins isolated or tested was determined by procedures described by us in detail in a previous paper (5). A concentration of 2.5 μg/plate of 2AN was used in all cases; initial concentration of test substance was 600 μg, and in some cases (toxicity or activity determinations) concentrations of 300 and 150 μg were also assayed. In a few cases the inhibitory action of certain coumarins was studied on other mutagens: e.g., acetylaminofluorene (AAF) and benzo[*a*]pyrene (B[*a*]P), which require metabolic activation with the Ames S-9 enzyme preparation (6), and 2-nitrofluorene (2NF), which does not require metabolic activation. The concentrations (μg/plate) of various mutagens were 25 (AAF), 1.0 (B[*a*]P), and 2.5 (2NF), respectively. The average numbers of colonies of positive controls (i.e., no test substance present) were: 2AN 2500, AAF 743, B[*a*]P 351, and 2NF 533. In most cases the colonies were counted after 72-h incubation at 37.5°. Toxicity determinations (only for 2AN studies) were conducted in the absence of test substance but in the presence of histidine and S-9 (5).

GENERAL ISOLATION AND CHARACTERIZATION PROCEDURES.—Melting points were determined on a Kofler hotstage microscope and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were obtained with a Bruker WM250 spectrometer using TMS as internal standard. High resolution mass spectra were obtained with an AEI MS-902 instrument. Uv spectra were obtained in MeOH with a Varian 2290-UV-VIS spectrometer, ir spectra with a Perkin-Elmer 467 Grating spectrometer. Standard chromatography was carried out on Si gel E Merck 230–240 mesh or Baker Flash chromatography Si gel using in general CH_2Cl_2 as eluent with a gradient of 0.5–10% MeOH, collecting 15-ml fractions with an automatic fraction collector. For tlc determinations precoated Si gel plates were utilized: normal phase, EM precoated Si gel 60, F254, usual solvent 10% MeOH in CH_2Cl_2; reversed phase, Baker precoated Si gel C_{18}-F plates, usual developer 5–

[1]For Part 2 in this series see Wall *et al.* (1).

10% H_2O in MeOH. Exposure of plates to iodine vapor was used as a general detection agent; alternatively, spraying with phosphomolybdate reagent followed by heating was utilized.

Psoralea corylifolia L. (Fabaceae), India.—The extraction and chromatographic profile of an extract of the seeds of this plant were described in detail in a previous report (1). After Si gel chromatography, a relatively nonpolar fraction was obtained that contained as the major constituent the phenolic terpene, bakuchiol (1). Further chromatography of the crude bakuchiol fraction on Si gel in C_6H_{14}-CH_2Cl_2 (1:1) yielded 1.3 g of bakuchiol and 0.068 g of a fraction which on crystallization from aqueous MeOH gave a pure component (mp 157–158°). The hrms of this compound showed $[M]^+$ to be 186.0319 (calcd for $C_{11}H_6O_3$, 186.0317). The ir, uv, and particularly the ^{1}H-nmr spectrum which showed the characteristic coupled furan doublets at δ 6.83 and 7.80 were identical to that of the known furanocoumarin, psoralen [**3**] (8,9). Psoralen inhibited the mutagenic activity of 2AN toward *S. typhimurium*. The inhibitory activity (IA) was 60% at 600 μg/plate. It was nontoxic at 600- and 300-μg dose levels in the absence of mutagen and presence of histidine and S-9 (6).

Selinum monniere (L.) Cusson (Apiaceae), China.—A CH_2Cl_2 extract of seeds of *S. monniere* consistently showed very high IA in the 2AN assay. After concentration of the CH_2Cl_2 extract, the residue was partitioned between 90% MeOH/10% H_2O and petroleum ether (1:1). The inhibition activity remained in the 90% MeOH layer. After concentration, this fraction was chromatographed on 80 g of Baker Si gel. One hundred 15-ml fractions were collected eluting with 95% C_6H_{14}/5% Et_2O and 85 15-ml fractions eluting with 90% C_6H_{14}/10% Et_2O. Fractions were combined according to tlc patterns obtained using the solvent system 99.5% CH_2Cl_2/0.5% MeOH. The fractions obtained in the more polar eluent 1610–1750 ml have similar tlc patterns and contained most of the total material collected by chromatography. This fraction, R_f of major spot 0.75, was strongly inhibitory in the 2AN assay. The crude active fraction was crystallized from EtOH/Et_2O to yield a pure compound (mp 105°). The hrms showed $[M]^+$ to be 270.0892 (calcd for $C_{16}H_{14}O_4$, 270.0884). The ir, uv, and ^{1}H-nmr spectra were identical to that of the known linear furanocoumarin imperatorin [**4**] (10,11). Imperatorin showed high IA (71% at 0.3 and 57% at 0.15 mg) and was nontoxic at these concentrations. From the mother liquors of the crystallization of imperatorin, another coumarin was isolated (mp 80–82°). The hrms showed $[M]^+$ to be 244.1099 (calcd for $C_{15}H_{16}O_3$, 244.1098). The ir, uv, and ^{1}H-nmr spectra were identical with the known coumarin osthol [**5**] (12). Osthol showed IA of 68 and 42% at 600 μg/plate in successive assays and was nontoxic at doses of 600 and 300 μg.

RESULTS AND DISCUSSION

The structures of the coumarins and the percentage inhibition of the mutagenicity of 2AN toward *S. typhimurium* (T-98) by the coumarins isolated and those available for comparison purposes are shown in Table 1. It is evident that coumarins with the simple benzopyrone ring system such as **1** or **2** are relatively inactive in the 2AN antimutagenic assay. On the other hand, osthol [**5**], which bears an 8-prenyl (dimethylallyl) substituent, shows higher activity. In general, linear furanocoumarins, particularly **4** which also bears an 8-*o*-prenyl substituent, were more active. All of the coumarins **1**–**7** were nontoxic at 600 μg/plate. Table 2 compares the mutagenic inhibition of selected coumarins on four mutagens, 2AN, AAF, B[*a*]P, and 2NF. Of these, the first three require metabolic activation. It is evident, as was noted in the case of our previous flavonoid study (1), that activity varied both with the structure of the coumarin and the mutagen. In general, the greatest inhibition was noted with the mutagen B[*a*]P in which system imperatorin [**4**] was very active, even at the lowest dose range. The compounds **4** and **5**, in general, were the most active of all the mutagens tested.

In contrast to the prenylated flavonoids, which were found to be very toxic in our previous study, the prenylated coumarins were both quite active and nontoxic. Hence, their activity may be either desmutagenic or antimutagenic (bioantimutagenic). Kada *et al.* (13) have applied the former term to indicate factors directly interacting with mutagens before affecting cells: the latter term was applied to factors which modulate mutagenesis at the cellular level. With the data on hand we are unable to make any mechanistic predictions. The problem of our investigations is further complicated by the fact that in most cases (i.e., with 2AN, AAF, and B[*a*]P) the mutagens require metabolic activation. Hence, the inhibition of the mutagenic activity could occur by

TABLE 1. Structure and Inhibition of 2-Aminoanthracene (2AN) Mutagenicity by Various Coumarins.

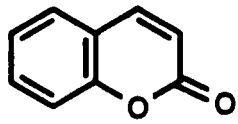
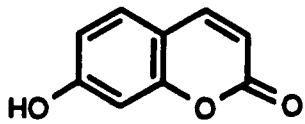
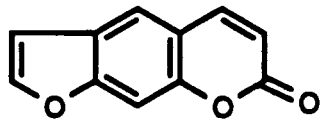
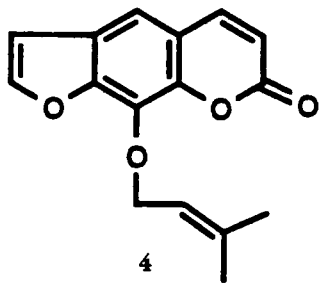
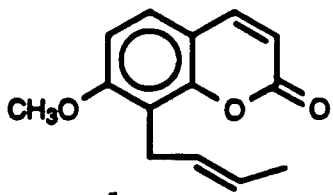
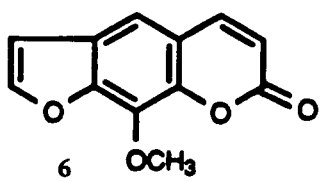
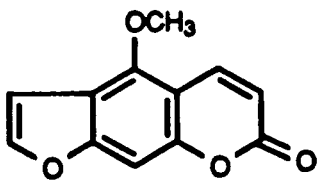
Compounds	Structure	% Inhibition of 2AN Mutagenicity (Dose, 600 μg/plate)
Coumarins Coumarin	 1	22%
Umbelliferone	 2	14%
Psoralen[a]	 3	60%
Imperatorin[a]	 4	81%
Osthol[a]	 5	68%
8-Methoxypsoralen	 6	44%

TABLE 1. Continued.

Compounds	Structure	% Inhibition of 2AN Mutagenicity (Dose, 600 μg/plate)
5-Methoxypsoralen	 7	23%

[a]Isolated at Research Triangle Institute.

interaction of a coumarin with the mutagen or with the mutagen metabolite as was noted by Wood *et al.* (14) in the case of the action of ellagic acid on a B[*a*]P metabolite. Moreover, the coumarin could affect the crude P-450 enzyme system, or the coumarin itself could be metabolized to a more active form.

The value of the 2AN mutagenic inhibition assay procedure for rapidly screening the plant kingdom and assaying chromatographic fractions leading to the isolation of potential antimutagenic agents is again borne out in this investigation.

Because of previous studies indicating that coumarin [**1**] showed inhibition of carcinogenesis (3) and desmutagenic or antimutagenic activity (4), and in view of our current studies with a number of other coumarins which show a combination of nontoxicity and very high activity in the inhibition of B[*a*]P mutagenesis (Table 2), we believe that a much more extensive investigation into this group of compounds is warranted.

TABLE 2. Percent Inhibition of Mutagenic Activity by Various Coumarins.

Coumarins	Micrograms mutagen/plate											
	2AN[a]			AAF[b]			B[*a*]P[c]			2NF[d]		
	600	300	150	600	300	150	600	300	150	600	300	150
Coumarin [**1**]	22	0	0	36	28	5	53	16	10	73	17	24
Imperatorin [**4**][e]	81	0	0		56	35	90	87	86	58	43	22
Osthol [**5**][e]	68	0	0	74	56	51	54	49	13	80	55	29
8-Methoxypsoralen [**6**]	44	0	0	39	21	0	86	76	58	25	39	5
5-Methoxypsoralen [**7**]	23	0	0	0	0	0	75	67	61	40	41	27

[a]2AN, 2-aminoanthracene.
[b]AAF, acetylaminofluorene.
[c]B[*a*]P, benzo[*a*]pyrene.
[d]2NF, nitrofluorene.
[e]Isolated at Research Triangle Institute.

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