with a 100-fold excess of M-MuLV 35*S* RNA.

Many of the DNA circles with a molecular weight of 5.5×10^6 now appear to contain single-stranded tails. None of the nicked SV40 molecules, present in the same mixture in 100-fold excess, had any detectable tails. These single-stranded tails have not been well characterized. They may be a mixture of viral RNA and strands of viral DNA partially displaced from the DNA duplex by the annealed viral RNA. They have only been observed after annealing with viral-specific RNA. These tails were derived from the presence of viral RNA and thus constitute additional evidence of the viral origin of the DNA with a molecular weight of 5.3×10^7 to 5.7×10^6.

Electron microscope observation has been necessary to determine the purity of the proviral DNA. The chemically pure proviral DNA should now make possible detailed characterization of its structure.

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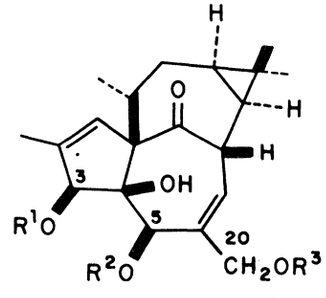
Antileukemic Principles Isolated from Euphorbiaceae Plants

Abstract. *Extracts of* Euphorbia esula *L. and* Croton tiglium *L., two members of the Euphorbiaceae which have been used widely in folk medicine for treating cancers, showed antileukemic activity against the P-388 lymphocytic leukemia in mice. Systematic fractionation of the extract of* Euphorbia esula *L. led to characterization of a major antileukemic component as the new diterpenoid diester, ingenol 3,20-dibenzoate. Similar fractionation of* Croton *oil led to characterization of phorbol 12-tiglate 13-decanoate as an active principle.*

Plants of the family Euphorbiaceae have been used to treat cancers, tumors, and warts from at least the time of Hippocrates (circa 400 B.C.), and references to their use have appeared in the literature of many countries (*1*). We report the isolation and characterization of antileukemic principles from *Euphorbia esula* L. and *Croton tiglium* L., two members of the Euphorbiaceae which are among those used widely in folk medicine (*1*).

In the course of our search for tumor inhibitors from higher plants, we found that an alcohol-water (95 : 5) extract (at room temperature) of *Euphorbia esula* L. from Wisconsin (*2*) showed significant inhibitory activity when tested in rodents against the sarcoma 180, Walker 256 carcinosarcoma, Lewis lung carcinoma, and P-388 lymphocytic leukemia (*3*). The fractionation of an active extract led to the characterization of a major antileukemic component as the new diterpene diester, ingenol dibenzoate (**1**). Ingenol dibenzoate shows significant inhibitory activity, at dosages of 130 to 360 μg per kilogram of body weight, against the P-388 lymphocytic leukemia (*3*).

Successive solvent partition of the alcoholic extract of *E. esula* led to concentration of the antileukemic (P-388) activity in the chloroform layer of a chloroform-water partition. Column chromatography of the residue from the chloroform solution on SilicAR CC-7 and subsequent thin-layer chromatography (TLC) on ChromAR were guided by testing for P-388 inhibitory and piscicidal activity. This procedure led to the isolation of ingenol dibenzoate (**1**, 0.0002 percent of the plant weight), a resinous material (*4*) with specific optical rotation at 28°C for the sodium D line ($[\alpha]_D^{28}$) + 268° (*c*, 0.0026, ethanol). High-resolution mass spectrometry (HRMS) (chemical ionization: methane reagent gas) indicated the empirical formula to be $C_{34}H_{36}O_7$ [calculated ($M^+ + H$), 557.252; found, 557.254].



1 $R^1 = R^3 = COC_6H_5$, $R^2 = H$
2 $R^1 = R^2 = R^3 = H$
3 $R^1 = R^2 = R^3 = COCH_3$
4 $R^1 = R^3 = COC_6H_5$, $R^2 = COCH_3$

Spectral data (*5*) suggested that the active principle was a diterpene diester. Accordingly, methanolysis of **1** yielded the tetracyclic diterpene ingenol (**2**), identified by comparison of its spectra with published data (*6*). Acetylation of the methanolysis product afforded the triacetate (**3**; $C_{26}H_{34}O_8$; melting point, 195° to 197°C), which had identical physical constants with those reported (*6*) for ingenol triacetate. The nuclear magnetic resonance (NMR) spectrum of **1** possessed two A_2B_2X systems centered at τ 2.3, indicating the presence of two benzoate groups. This was supported by the mass spectrum of **1**, which contained a strong peak at mass/charge (*m/e*) 105 (C_6H_5CO).

The structural problem which remained at this point was the determination of the sites of attachment of the two benzoate groups to the ingenol ring system. The NMR signals for H-3 and H_2-20 of **1** appeared at lower magnetic field by 1.46 and 0.82 parts per million, respectively, than the corresponding signals of ingenol (*7*). Acetylation of **1** gave a monoacetate (**4**), and the NMR spectrum of **4** showed the H-5 signal at τ 4.42. These results indicated C-3 and C-20 to be the points of attachment of the two benzoate ester groups. Thus, compound **1** was proved to be ingenol 3,20-dibenzoate.

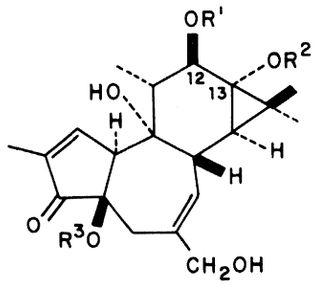
Our success with *E. esula* prompted us to search for tumor inhibitory principles in an extract of the seeds of a related genus. *Croton* oil, which is the seed oil of *Croton tiglium* L., a leafy shrub native to Southeast Asia, is commercially available (*8*). The diterpenoid esters of *Croton* oil are of current interest as a result of their demonstrated biological activity as cocarcinogens (*9*). When *Croton* oil was evaluated for possible effects on the P-388 lymphocytic leukemia in mice, significant inhibitory activity was noted (*3*). Fractionation of the oil led to the characterization of a major component as the known phorbol diester, phorbol 12-tiglate 13-decanoate (**5**). Com-

ponent **5** exhibits significant inhibitory activity at dosages of 60 to 250 μg per kilogram of body weight against the P-388 leukemia in mice (*3*).

The tumor inhibitory principles of *Croton* oil were divided into two active fractions on solvent partition between 10 percent aqueous methanol and Skellysolve B. Column chromatography of the residue from the aqueous methanol solution over SilicAR CC-7 deactivated by water and subsequent TLC and high-pressure liquid chromatography were guided in a manner analogous to that described for *E. esula*. This procedure led to the isolation of an active principle as a resinous material (*4*) (0.048 percent of the weight of *Croton* oil) with specific optical rotation at 27° for the sodium D line ($[\alpha]_D^{27}$) + 39° (*c*, 0.78, dioxane); HRMS showed the molecular ion at *m/e* 600.3550 (calculated, 600.3662). Comparison of the $[\alpha]_D$, ultraviolet, infrared, NMR, and mass spectra with those described for phorbol 12-tiglate 13-decanoate (*10*) indicated that the active constituent was **5**.

A series of commercially available (*8*) *Croton* oil principles (**6** to **9**) were assayed against P-388 lymphocytic leukemia in order to determine the potential significance of the ester side chains and of other structural features of compounds similar to phorbol. When these materials were assayed in parallel with **5**, only phorbol 12-tiglate 13-decanoate showed antileukemic activity over the dose ranges tested.

Also, in pursuing the antileukemic principles of several plants of the family Thymelaeaceae, we have isolated several diterpenoid esters that have considerable chemical similarity to the newly characterized principles of the Euphorbiaceae (*11*). In view of our earlier findings (*11, 12*), it will be of interest to determine the significance of various structural features for the antileukemic activity of the diterpenoid esters. Such studies may clarify also the paradoxical similarity in structure between the cocarcinogenic and antileukemic principles of the Euphorbiaceae and the Thymelaeaceae.



5 R^1 = CO - $(CH_3)C = CHCH_3$; R^2 = CO - $(CH_2)_8$ - CH_3; R^3 = H

6 $R^1 = R^2 = R^3$ = H

7 R^1 = CO - $(CH_2)_{12}$ - CH_3; R^2 = $COCH_3$; R^3 = H

8 R^1 = CO - $(CH_2)_{12}$ - CH_3; R^2 = $COCH_3$; R^3 = CH_3

9 $R^1 = R^2$ = COC_6H_5, R^3 = H

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4. The homogeneity of the material was confirmed by TLC with several solvent systems and by high-pressure liquid chromatography.
5. Additional physical constants for **1** are: ultraviolet absorption maximum (ethanol) (log ε), 230 (4.46), 272 (3.37), 280 (3.31) nm; infrared absorption maximum (chloroform), 2.86, 3.45, 3.52, 5.82, 5.85, 7.88 μm; nuclear magnetic resonance spectrum (deuterochloroform) τ 8.96 (3H, d, H_3-18), 8.93 (6H, s, H_3-16,-17), 8.16 (3H, br s, H_3-19), 6.02 (1H, s, H-5), 5.84 (1H, dd, *J* = 11, 4 hertz, H-8), 5.09 (2H, AB q, H_2-20), 4.22 (1H, s, H-3), 3.87 (1H, br s, H-1), 3.76 (1H, d, *J* = 4 hertz, H-7), 2.5 (6H, m, two of B_2X portion of A_2B_2X, *m*- and *p*-benzoate protons), 1.96 (4H, two of A_2 portion of A_2B_2X, *o*-benzoate protons); mass spectrum *m/e* 556 (M^+), 538, 434, 416, 312, 294.
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Representation of the Visual Field on the Medial Wall of Occipital-Parietal Cortex in the Owl Monkey

Abstract. *The medial visual area is located on the medial wall of occipital-parietal cortex. A much larger proportion of this area is devoted to the representation of the more peripheral parts of the visual field than in any other cortical area or subcortical visual structure that has been mapped previously in any species of primate.*

In the representations of the visual field in almost all of the cortical visual areas and in the subcortical visual structures in primates, a very large proportion of each visuotopic map is devoted to the representation of the small central part of the visual field in which primates see with high acuity (*1*). Described in this report is the single known exception to this rule, the medial visual area, which is located on the medial wall of occipital-parietal cortex. A much greater proportion of the medial area is devoted to the representation of the relatively more peripheral parts of the visual field than in any other visuotopically organized cortical area or in any subcortical visual structure, including the lateral geniculate nucleus, inferior pulvinar, or superior colliculus, that has been mapped in any primate. Each of these representations of the visual field is likely to perform its own set of functions in the analysis and integration of visual information, and in whatever functions that are performed by the medial area there appears to be a considerably greater emphasis on input from the more peripheral parts of the visual field.

This study is part of a series of investigations in which we have sought to identify and determine the organization of the major functional units, the neural representations of the visual field, in the visual system of primates. In most of these investigations, we have explored the cerebral cortex because this structure contains the great bulk of the neurons involved in the processing of visual information. The owl monkey (*Aotus trivirgatus*) was chosen as our experimental animal because the cerebral cortex is relatively less convoluted in this species than in most other simian primates, thus facilitating our task of mapping the representations of the visual field in the cortex. In this part of the total