Actinomycin D given 1 hour after the rat is exposed to cold does not affect the subsequent rise in ODC activity in the adrenal medulla, whereas it does in the adrenal cortex. Concurrently, the rise in ODC activity is later in the cortex (Fig. 1, A and B) than in the medulla. This delay in the increase in ODC activity in the cortex as compared to the medulla appears to reflect a temporal difference in the synthesis of new mRNA. The proposed mechanism for the mediation of ODC activity by cyclic AMP involves the activation of protein kinase. Guidotti and Costa have recently observed that there is a rapid stimulation of cyclic AMP–dependent protein kinase activity in the adrenal medulla after cold exposure (*11*). These data plus the previously reported observations that injection of dibutyryl cyclic AMP results in large increases in ODC activity in the adrenal glands (*7*) and in the liver (*12*) of the rat suggest that ODC activity may be regulated by cyclic AMP.

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References

1. D. Russell and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420 (1970).
2. ———, *Endocrinology* **84**, 223 (1969); ———, V. Medina, *ibid.* **86**, 1414 (1970); D. H. Russell and R. L. Taylor, *ibid.* **88**, 1397 (1971); W. Panko and F. Kenney, *Biochem. Biophys. Res. Commun.* **43**, 346 (1971); A. Pegg and H. G. Williams-Ashman, *Biochem. J.* **109**, 32 (1968).
3. D. H. Russell and S. H. Snyder, *Mol. Pharmacol.* **5**, 253 (1969).
4. A. Guidotti and E. Costa, *Science* **179**, 902 (1973); A. Guidotti, B. Zivkovic, R. Pfeiffer, E. Costa, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **278**, 195 (1973); E. Costa and A. Guidotti, *New Concepts in Neurotransmitter Regulation*, A. J. Mandell, Ed. (Plenum, New York, 1973), p. 135; ———, I. Hanbauer, *Life Sci.* **14**, 1169 (1974).
5. C. C. Mao and A. Guidotti, *Anal. Biochem.* **59**, 63 (1974).
6. J. Kuo and P. Greengard, *Adv. Cyclic Nucleotide Res.* **2**, 41 (1972).
7. R. Richman, C. Dobbins, S. Voina, L. Underwood, D. Mahaffee, H. J. Gitelman, J. Van Wyk, R. L. Ney, *J. Clin. Invest.* **52**, 2007 (1973).
8. M. M. Appleman, W. J. Thompson, T. R. Russell, *Adv. Cyclic Nucleotide Res.* **3**, 65 (1973).
9. G. M. Walton, G. N. Gill, I. B. Abrass, L. D. Garren, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 880 (1971); C. Eil and I. G. Wool, *Biochem. Biophys. Res. Commun.* **43**, 1001 (1971).
10. T. A. Langan, *Ann. N.Y. Acad. Sci.* **185**, 166 (1971); V. M. Kish and L. J. Kliensmith, *J. Biol. Chem.* **249**, 750 (1974).
11. A. Guidotti and E. Costa, personal communication.
12. W. T. Beck, R. A. Bellantone, E. S. Canellakis, *Biophys. Biochem. Res. Commun.* **48**, 1649 (1972); *Nature (Lond.)* **241**, 275 (1973).
13. L. D. Garren, R. L. Ney, W. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **53**, 1443 (1965).

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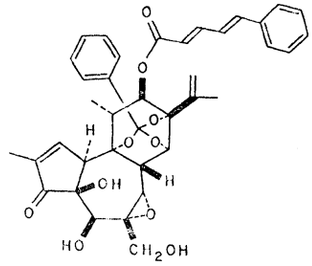
Mezerein: Antileukemic Principle Isolated from *Daphne mezereum* L.

Abstract. *An alcohol-water extract of* Daphne mezereum *L., a plant widely used in folk medicine for treating cancers, showed antileukemic activity against the P-388 lymphocytic leukemia in mice. Systematic fractionation of the extract has led to isolation and characterization of mezerein as the principal antileukemic component.*

Daphne mezereum L. and other *Daphne* species (family Thymelaeaceae) have been used to treat cancers from the time of Aphrodisias (circa A.D. 200), and references to their use in folk medicine have appeared in the herbal literature of many countries (*1*).

In the course of our search for tumor inhibitors of plant origin, an alcohol-water (1 : 1) extract of the seeds of *Daphne mezereum* L. from Italy (*2*) showed significant inhibitory activity when tested in mice against the P-388 lymphocytic leukemia (*3*). We report herein the fractionation of an active extract and the characterization of the principal antileukemic component, which is identified as mezerein. Mezerein shows significant inhibitory activity, at dosages of 50 μg per kilogram of body weight, against the P-388 and L-1210 leukemias in mice (*3*).

Successive solvent partition of the chloroform extract of the ground seeds of *D. mezereum* led to concentration of the antileukemic (P-388) activity in the chloroform layer of a chloroform-water partition and the aqueous methanol layer of a 10 percent aqueous methanol–Skellysolve B partition. Column chromatography of the residue from the aqueous methanol solution on SilicAR CC-7 and subsequent thin-layer chromatography (TLC) on Chromar were guided by testing for antileukemic (P-388) activity and goldfish toxicity. This procedure led to the concentration of the active principle (or principles) into a single TLC-homogeneous fraction (0.05 percent of the plant weight). Crystallization from dichloromethane–diethyl ether afforded the principal active constituent as colorless prisms with melting point (mp) 258° to 262°C and specific optical rotation at 27°C for the sodium D line ($[\alpha]_D^{27}$) + 125° in chloroform. The mass spectrum showed the molecular ion (M^+) at *m/e* (mass/charge) 654. Ultraviolet absorption maxima in ethanol [and log extinction (log ε)] were 314 (4.63), 240.5 (4.23), 233.5 (4.26), and 227 (4.23) nm. A comparison of the mp, $[\alpha]_D$, ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectra with those described for mezerein (*4*)



indicated that the active constituent was mezerein. Confirmatory evidence was secured by methanolysis of the active compound, which yielded 12-hydroxydaphnetoxin, identified by comparison of its spectra with published data (*4*, *5*), and methyl 5-phenyl-2,4-pentadienoate (*6*), identified by comparison (mixture mp, gas-liquid chromatography, and IR, UV, and NMR spectra) with a sample prepared by synthesis.

Also, in pursuing the antileukemic principles of several *Gnidia* species (Thymelaeaceae), we have recently characterized three new agents from *G. lamprantha* Gilg (gnididin, gniditrin, and gnidicin) which are close chemical relatives of mezerein (*7*). In view of our earlier findings (*7*, *8*), it will be of interest to determine the significance of the ester, the epoxide, the cyclopentenone, the orthoester, and of other structural features for the antileukemic activity of mezerein-like compounds.

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References and Notes

1. J. L. Hartwell, *Lloydia* **3**, 204 (1971).
2. Seeds of *Daphne mezereum* L. were collected in Italy in November 1966. We thank R. E. Perdue, Jr., of the U.S. Department of Agriculture, Beltsville, Maryland, for supplying the plant material.
3. Antileukemic activity was assayed under the auspices of the National Cancer Institute as described by R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott [*Cancer Chemother. Rep. Part 3* **3**, 1 (1972)]. Evaluation of assay results on a sta-

tistical basis in sequential testing is such that a material is considered active if it causes an increase in survival of treated animals (T) over controls (C) resulting in $T/C \geq 125$ percent.
4. A. Ronlán and B. Wickberg, *Tetrahedron Lett.* (1970), p. 4261; H. Schildknecht and R. Mauser, *Chem. Zt. Chem. App.* **94**, 849 (1970).
5. J. Coetzer and M. J. Pieterse, *J. S. Afr. Chem. Inst.* **9**, 241 (1971).
6. F. W. Hinrichsen and W. Triegel, *Justus Liebigs Ann. Chem.* **336**, 198 (1904).
7. S. M. Kupchan, J. G. Sweeny, R. L. Baxter, T. Murae, V. A. Zimmerly, B. R. Sickles, *J. Am. Chem. Soc.*, in press.
8. S. M. Kupchan, *Fed. Proc.* **33**, 2288 (1974).
9. This communication is part 103 in the series entitled "Tumor Inhibitors"; part 102 is (*7*). Supported by grant CA-11718 and contract NO1-CM-12099 from the National Cancer Institute and grant CI-102J from the American Cancer Society.

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Gene Transfer to a Myxobacterium by Escherichia coli Phage P1

Abstract. Myxococcus xanthus *is a bacterium with an interest for studies of development because it has an organized multicellular phase in its life cycle. Bacteriophage P1 can adsorb to* M. xanthus *and inject its DNA into this organism despite the wide taxonomic gap separating myxococcus from* Escherichia coli, *the source of P1. A specialized transducing derivative of P1, called P1CM, can carry a gene for chloramphenicol resistance from* E. coli *into* M. xanthus *and generate unstable drug-resistant strains.*

Fruiting myxobacteria can propagate indefinitely as independent vegetative cells that have typical gram-negative bacterial morphology (*1*). However, when appropriately starved, myxobacterial cells stop dividing and aggregate to form a multicellular fruit of species-specific shape in which individual cells become spores (*2*). The behavior of myxobacteria during aggregation and fruiting shows a high degree of coordination between the actions of separate cells and suggests that they might be appropriate organisms in which to study communication between cells (*3*). To advance a combined biochemical and genetic attack on this problem we have sought a mode of genetic exchange, because none had yet been described for any of the myxobacteria. We now report that phage P1 can transfer genes to *Myxococcus xanthus*. P1 is a general transducing phage isolated from *Escherichia coli* Li that can infect many species of enteric bacteria (*4*). Because *Myxococcus* forms multicellular fruiting bodies, exhibits gliding motility, and has a DNA base composition of 60 to 70 percent guanine • cytosine pairs, it has been placed in a taxonomic group far from the enteric bacteria (*5*). Gene transfer between the two groups is, therefore, surprising.

When a drop of a P1 suspension was placed on the surface of an agar plate seeded with cells of *M. xanthus* strain FB, bacterial growth under the drop was inhibited and a zone of clearing was produced. Five different stocks of P1 were tested and all produced clearing. Material from a clear zone, suspended and placed on a fresh bacterial lawn, did not produce clearing. The titer of P1 in this material (measured on *E. coli* K12) was no greater than that expected for dilution of the drop into the agar, that is, no phage production was detectable. To test whether clearing was due to the phage itself or to some other lytic agent, lysozyme for example, a P1 lysate was sedimented to equilibrium in CsCl and the phage-containing band was isolated. This purified P1 had the same clearing activity as the original lysate.

P1 adsorbed efficiently to *M. xanthus* cells. When P1 was mixed with FB cells at a density of 2×10^8 per milliliter, suspended in a solution of 2 percent Bacto-Casitone containing 2.5 mM $CaCl_2$ and 0.1 mM $MgCl_2$, and incubated at 32°C for 15 minutes, more than 99 percent of the P1 was removed from the supernatant after sedimentation of cells. A cell-free supernatant of FB had some capacity to inactivate P1, but the rate of inactivation was 3.5 times greater in a cell suspension than in a cell-free culture supernatant.

Table 1. Transfer of chloramphenicol resistance to *M. xanthus* by P1CM. The indicated number of phage particles or the number of molecules of DNA extracted from phage with phenol (*13*) were mixed with 1.5×10^8 exponentially growing FB cells in a total volume of 0.3 ml of 2 percent Bacto-Casitone containing 2.5 mM $CaCl_2$, and incubated for 17 hours at 32°C with aeration. Finally the mixture was divided into four portions and plated on Casitone agar containing chloramphenicol (25 μg/ml). Colonies were counted after incubation at 30°C for 4 days. The total number of colonies for the four portions is reported.

Addition	Chloramphenicol-resistant colonies
7×10^9 P1CM particles	83
3.5×10^9 P1CM particles	36
3.5×10^9 P1 particles	<1
None	<1
8×10^9 P1CM DNA molecules	3

To test whether phage DNA entered the cells, phage P1CM was applied to strain FB. Phage P1CM carries a gene specifying chloramphenicol transacetylase from an R factor (*6*). Bacteria and P1CM were mixed, incubated, and plated on Casitone (*7*) agar containing chloramphenicol. As indicated in Table 1, chloramphenicol-resistant colonies of *M. xanthus* arose from the cultures containing P1CM, but not from controls that contained P1 rather than P1CM. The number of drug-resistant colonies obtained was proportional to the number of P1CM particles added, with an efficiency of about 10^{-8} resistant colonies per phage.

To test whether P1 injected its DNA into the cell or released it at the cell surface to be taken up by a transformation-like mechanism, DNA extracted from P1CM was added to FB. Although some chloramphenicol-resistant colonies were observed (last line of Table 1), there were 25 times fewer colonies than from an equivalent amount of phage. The more efficient transfer from phage as compared to transfer from free DNA favors (but does not prove) injection. Despite the taxonomic gap separating *M. xanthus* from *E. coli*, the ability of P1 to adsorb to DNA and its apparent ability to inject DNA into myxobacterial cells suggests the presence of related surface structures. Lipopolysaccharide is the adsorption receptor for P1 in *E. coli* (*8*), and recently *Myxococcus* was shown to possess lipopolysaccharide similar to that from *E. coli* and *Salmonella* (*9*).

Chloramphenicol-resistant bacteria that arose from P1CM infection of FB grew on both solid and liquid media containing 25 μg of chloramphenicol per milliliter. Cultures have been carried through serial single colony isolations, representing more than 60 cell generations, without loss of drug resistance. In spite of that, the chloramphenicol-resistant character of these bacteria is unstable, and colonies that formed on solid medium without chloramphenicol contain drug-sensitive cells. The growth rate of the chloramphenicol-resistant bacteria in chloramphenicol liquid medium was one-third that of parental cells without chloramphenicol. The chloramphenicol-resistant bacteria failed to produce P1 phage, detectable by