ine tissue, these observations indicate that, in both the 9.5*S* and 5*S* complexes, sulfhydryl groups of the receptor substances actually participate in the estrogen-binding phenomenon, perhaps indirectly by contributing to receptor structure or conformation.

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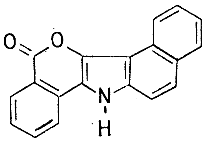
9. Tritiated estradiol also binds to a component of rat serum (probably albumin), giving rise to a peak of radioactivity likewise sedimenting in the region of 5*S*. This association, which is much weaker than the binding to uterine receptors, has been found by T. Suzuki in our laboratory to be unaffected by sulfhydryl-blocking reagents. In that homogenates of uteri contain appreciable amounts of serum proteins, comparison of the sedimentation pattern in the presence and absence of organic mercurials provides a convenient test to determine whether a 5*S* peak represents estradiol associated with the receptor from uterine nuclei or an artifact resulting from the interaction of free estradiol with a serum contaminant.
10. Supported by PHS research grant CA-02897 from the National Cancer Institute and a Ford Foundation fellowship grant (D.J.H.).

3 August 1967

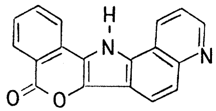
5-Oxo-5H-benzo[e]isochromeno[4,3-b] indole, a New Type of Highly Sarcomagenic Lactone

Abstract. *The title compound, which belongs to a new synthetic group of polycyclic lactones derived from isocoumarin, exhibits a remarkably high degree of carcinogenicity* in situ *when injected in mice. Cancer-inducing activity has also been found in similar isocoumarins, a family known to include many naturally occurring substances.*

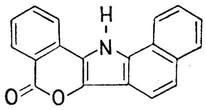
In recent years much attention has been paid to the local carcinogenic effects of natural (*1*) or synthetic (*2*) compounds bearing lactone groups, the most widely investigated being the polycyclic furanoid lactones of the aflatoxin family (*3*). We wish to record here the outstanding activity of 5-oxo-5*H*-benzo[*e*]isochromeno [4,3-*b*] indole (I), an entirely new type of polycyclic carcinogen in whose molecular structure a lactone function is present in the form of an isocoumarin system.



I



II



III

This substance, recently synthesized in our laboratory (*4*), was tested in two strains of mice, strain C3H and strain Swiss (Carshalton, Surrey, United Kingdom). It was administered by subcutaneous injection in the flank of 3- to 4-month-old male and female mice (three injections of 0.6 mg in 0.2 ml of neutral, sterile olive oil, 1 month elapsing between each injection). The results, recorded in Table 1, evidence the remarkable speed and magnitude of the action of compound I, which rank it among the most potent carcinogenic agents known.

Carcinogenicity was also found, although to a lesser degree, in another nitrogenous coumarin similar to I, namely 8-oxo-8*H*-isochromeno[4′,3′:4,5] pyrrolo[2,3-*f*]quinoline (II); tested in mice of C3H strain (14 male and 14 female) in the same conditions as for I, this substance gave one sarcoma in two males after 309 days, and one sarcoma in two females after 282 and 296 days respectively. Further, carcinogenicity in this family of lactones is highly dependent on chemical structure, as witnessed by the total inactivity of 5-oxo-5*H*-benzo[*g*]isochromeno[4,3-*b*] indole (III), which although isomeric with I, had not elicited any tumors in 14 male and 14 female mice (C3H strain) after 450 days.

These findings have a twofold significance. First, compounds such as I and II represent an entirely new class of carcinogens, which can be considered as structurally hybrid between the current polycyclic carcinogens (hydrocarbons, benzacridines, benzocarbazoles) and the lactones. Second, that such a highly carcinogenic substance as I should be found in the chemical family of isocoumarins is of particular importance in view of the presence of the isocoumarin ring in the molecule

Table 1. Sarcomagenic effect of compound I.

Strain C3H				Strain Swiss			
Male		Female		Male		Female	
Survival (days)	Sarcoma on day:	Survival (days)	Sarcoma on day:	Survival (days)	Sarcoma on day:	Survival (days)	Sarcoma on day:
140	90	115	82	103	78	150	102
140	90	115	82	103	78	150	102
140	90	117	89	110	82	150	102
140	101	117	89	112	82	150	102
140	107	117	89	149	102	153	112
140	107	117	89	149	102	153	112
194	152	117	89	149	109	153	112
201	152	117	92	149	109	153	112
241	186	117	92	176	136	153	122
256	186	117	92	176	136	157	122
363	302	167	129	183	142	176	142
373	302	174	132	183	142	176	142
450*		194	140			182	149
450		197	140			182	149

* Animals showing no tumor after 450 days were killed. Tested in over 600 mice of each sex and belonging to the same strains, the solvent used alone has never shown any sarcomagenic activity. In the present experiment the controls consisted of 28 mice (14 male and 14 female) of each strain.

of a variety of natural products, some of which, such as ellagic acid and its derivatives, are contained in vegetable foods (*5*). A search for carcinogenicity among isocoumarins thus seems to be indicated.

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Enthalpy of Transformation of a High-Pressure Polymorph of Titanium Dioxide to the Rutile Modification

Abstract. *The enthalpy of transformation of a high-pressure form of titanium dioxide, which has the alpha lead dioxide structure, to the rutile modification was measured by the method of "transposed temperature-drop calorimetry." For the reaction, titanium dioxide (alpha lead dioxide form) transforming to rutile, the change in the heat content is* -0.76 ± 0.17 *kilocalorie per mole. From this value and the volume change (+ 2.8 percent) associated with the transformation, we estimate the equilibrium pressure at 294°K to be* 60 ± 20 *kilobars.*

High-pressure studies (*1, 2*) have shown the existence of a new high-pressure polymorph of titanium dioxide (TiO_2) which possesses the orthorhombic alpha PbO_2 structure and has a density about 2.8 percent greater than that of rutile. This material has been recovered as a metastable product from rutile single crystals shocked to pressures greater than 330 kb (*1*). It also has been synthesized directly from rutile at pressures of 40 to 120 kb and temperatures of 400° to 1500°C (*2*). This is not in contradiction to the x-ray studies to 150 kb (*1*) in which no conversion was found, in that for kinetic reasons the reaction probably does not occur at room temperature. Similarly, the α-PbO_2 transformation is bypassed under explosive loading on compression. At atmospheric pressure, this metastable form of TiO_2 transforms rapidly to rutile at temperatures above about 450°C (*1*).

To elucidate the stability of this phase, we have measured its enthalpy of transformation to rutile by means of a novel technique, "transposed temperature-drop calorimetry." This method was used in this laboratory in order to determine the enthalpy of transformation of stishovite to silica glass (*3*).

In this calorimetric technique, a sample of the metastable high-pressure polymorph, contained in a thin-walled platinum capsule, is dropped from room temperature into a calorimeter maintained at a temperature high enough for rapid transformation to the low-pressure form. The observed heat effect in the calorimeter represents the algebraic sum of the heat content of the sample and its enthalpy of transformation to the low-pressure form at the calorimetric temperature. In a second calorimetric experiment on the same capsule, which now contains transformed material, the heat content of the transformed sample is obtained at the same temperature. The difference between the heat effects measured in the first and second experiments yields the enthalpy of transformation at room temperature.

The specimen of TiO_2 in the α-PbO_2 modification used in this work had been recovered from the single-crystal shock experiments of McQueen, Jamieson, and Marsh (*1*). It consisted of a coarse, crystalline, grayish powder with particles ranging in size from about 0.1 to 2 mm. The x-ray diffraction patterns of this sample indicated the presence of 5 to 10 percent of untransformed rutile as a second phase. Four separate, clean, dry, platinum capsules, each weighing about 120 mg, were filled with about 150 mg of sample which had been dried for 1 hour at 120°C with no observable loss in weight. The capsules were crimped shut by means of a three-jaw chuck.

The calorimeter and the experimental arrangements have been described (*3*). As before, calibration of the calorimeter (at 692°C) was achieved by dropping 2-mm wire platinum pieces into it from room temperature (21.0°C). The calibrating heat effects were calculated from the known weight of platinum and the equation for heat content of platinum given by Kelley (*4*). This equation was also used to calculate the heat content of the platinum capsules used. The total heat effect involved in each experiment was about 20 to 25 calories. Of this amount, about 3 calories, or some 15 percent, was due to the heat content of the platinum capsules. The enthalpy of transformation represented about 5 percent of the total heat effect.

The results obtained in the two sets of drop experiments are given in Table 1. Our mean value for the heat content (H) of rutile at 965°K is in agreement with the value (11.090 kcal/mole) calculated from the data of Kelley and Mah (*5*). From the difference in the mean heat effects observed in the two types of experiments, for the reaction

$$\mathrm{TiO_2\ (\alpha\text{-}PbO_2\ form) = TiO_2\ (rutile)} \qquad (1)$$

we calculate

$$\Delta H^{\circ}_{294} = -0.76 \pm 0.17\ \mathrm{kcal/mole}$$

This value is subject to two main uncertainties. We noted above that the available sample contains 5 to 10 percent rutile as a second phase. Correction of the calorimetric data for the presence of rutile would make the enthalpy of transformation according to reaction 1 more exothermic by some 0.04 to 0.08 kcal/mole. The second uncertainty arises from the strain energy stored in the shocked specimens. The broadened lines and the absence of back reflections in the x-ray diffraction patterns indicate that the specimens were in a highly strained condition. When the specimens transform spontaneously to rutile at high temperature, the major part of this strain energy is released along with the enthalpy