Technical Papers

The Absence of Dietary Effects on the DNA Content of Liver Nuclei of the Adult Rat

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Ely and Ross (*1*) reported recently that in young growing rats fed on protein-deficient diets for periods of 8–49 days, the liver nuclei had a higher mean desoxyribonucleic acid (DNA) content than had nuclei obtained from livers of control animals fed on the stock diet.

In the adult rat, dietary variations do not seem to have similar effects. Campbell and Kosterlitz (*2–4*) found in a large number of rats that feeding of a protein-free diet for periods of up to 3 weeks and variations in the fat and choline contents of the diet had no influence on the DNA content of the liver. The only factors that appeared to determine the value of liver DNA were body weight and sex. Mandel, Jacob, and Mandel (*5*) showed that, when adult rats were fed on a protein-free diet for 50–75 days, no DNA was lost from the liver and the number of nuclei remained unchanged. In order to give further support to these findings, we have now determined the mean DNA contents of liver nuclei isolated by the method of Vendrely and Vendrely (*6*). Five male littermate pairs of rats with a mean initial body weight of 325 g were used, one member of each pair being fed on the stock diet and the other on a protein-free but otherwise adequate diet for 28 days. The mean DNA content was 10.8×10^{-6} μg/nucleus in the rats fed on the stock diet and 11.0×10^{-6} μg/nucleus in the rats fed on the protein-free diet. The standard error of the difference between the DNA values of the two groups was $\pm 0.48 \times 10^{-6}$.

The significance of these results is further underlined by the findings of Davidson and McIndoe (*7*) and of Mirsky and Kurnick (*8*). These authors found no change in the mean DNA content of rat liver nuclei after fasting, which in the experiments of Mirsky and Kurnick was continued until the livers had lost as much as half their original weight.

It would appear, then, that in the adult rat even such severe dietary conditions as prolonged fasting and prolonged consumption of protein-free diets have no significant influence on the mean DNA content of liver nuclei. The DNA content of the liver of the adult rat is thus a suitable standard of reference for those liver constituents which vary with altered dietary conditions. On the other hand, the findings of Ely and Ross (*1*) indicate that this is not the case in young growing rats, at least not if they are exposed to severe dietary deficiencies for long periods. Under these conditions, the normal growth of the liver is seriously interfered with, and changes in the DNA content of liver nuclei are therefore more likely to occur than in adult animals.

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Manuscript received October 11, 1951.

A New Class of Organic Fungicides

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A series of new compounds has been synthesized by the reaction of perchloromethyl mercaptan with the alkali metal salts of imides and amides. The purified products obtained from these reactions are, in nearly all cases, colorless, crystalline, odorless compounds and are readily obtained in yields ranging from 80 to 95%. In a cooperative testing program with Rutgers University, the compounds were found to possess

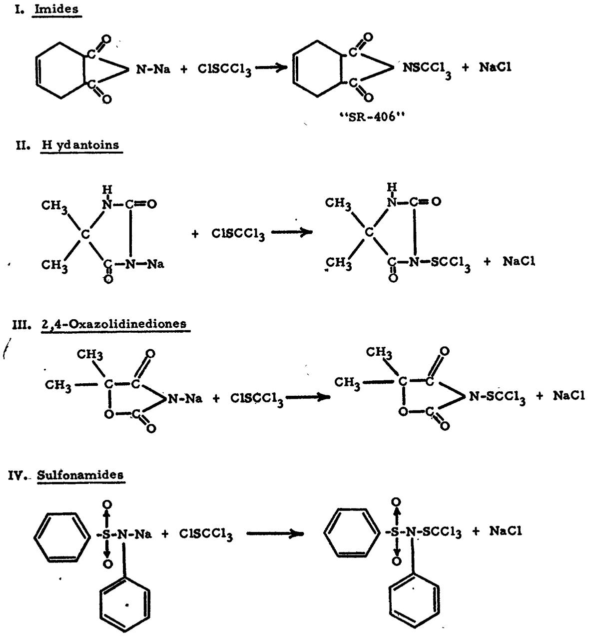

Fig. 1.

exceptional fungicidal activity. One of them, *N*-trichloromethylthio-tetrahydrophthalimide, which has undergone extensive fungicide field tests at numerous agricultural experimental stations in the United States, Canada, England, Denmark, France, and Latin America under the code identification "SR-406," is proving to be an especially promising agricultural fungicide. The results of these field tests will be reported elsewhere.

TABLE 1

N-TRICHLOROMETHYLTHIO DERIVATIVES OF COMPOUNDS CONTAINING THE > NH GROUP

Parent compound	Empirical formula	MP° C (uncorr)	Analysis (%)			
				N	S	Cl
Phthalimide	$C_9H_4NO_2SCl_3$	176–177	Found	4.72	10.64	35.76
			Calc	4.71	10.81	35.96
Δ4-Tetrahydrophthalimide	$C_9H_8NO_2SCl_3$	172–173	Found	4.79	10.59	35.00
			Calc	4.67	10.67	35.50
Endomethylene-Δ4-tetrahydrophthalimide	$C_{10}H_8NO_2SCl_3$	152–153	Found	4.36	10.40	33.67
			Calc	4.49	10.25	34.13
Succinimide	$C_5H_4NO_2SCl_3$	140–141	Found	—	13.00	41.69
			Calc	—	12.90	42.94
5,5-Dimethyl-2,4-oxazolidinedione	$C_6H_6NO_3SCl_3$	88– 89	Found	5.16	11.46	38.32
			Calc	5.02	11.49	38.13
5-Methyl-5-ethyl-2,4-oxazolidinedione	$C_7H_8NO_3SCl_3$	83– 84	Found	—	11.20	36.29
			Calc	—	10.95	36.35
5,5-Pentamethylene-2,4-oxazolidinedione	$C_9H_{10}NO_3SCl_3$	111–112	Found	—	10.24	33.31
			Calc	—	10.38	33.38
5-Methyl-5-isobutyl-2,4-oxazolidinedione	$C_9H_{12}NO_3SCl_3$	89– 90	Found	—	10.04	32.99
			Calc	—	10.00	33.18
5-Cyclopropyl-5-methyl-2,4-oxazolidinedione	$C_8H_8NO_3SCl_3$	108–109	Found	4.59	10.45	34.77
			Calc	4.79	10.79	36.49
O-Benzosulfimide	$C_8H_4NO_3S_2Cl_3$	143–144	Found	—	19.22	31.85
			Calc	—	19.27	32.08
N-Phenyl-benzenesulfonamide	$C_{13}H_{10}NO_2S_2Cl_3$	112–113	Found	—	18.02	27.59
			Calc	—	16.74	27.88
5,5-Dimethyl-hydantoin	$C_6H_7N_2O_2SCl_3$	169–171	Found	10.16	11.49	38.42
			Calc	10.11	11.55	38.45
1-Nitro-5,5-dimethyl-hydantoin	$C_6H_6N_3O_4SCl_3$	125–126	Found	12.35	9.85	32.74
			Calc	13.04	9.94	33.08
1-Acetyl-5,5-dimethyl-hydantoin	$C_8H_9N_2O_3SCl_3$	94– 97	Found	8.75	10.02	32.70
			Calc	8.78	10.03	33.40
5-Methyl-5-isobutyl-hydantoin	$C_9H_{13}N_2O_2SCl_3$	133–134	Found	8.61	9.72	33.00
			Calc	8.77	10.03	33.38
Morpholine	$C_5H_8NOSCl_3$	86– 87	Found	—	13.46	44.39
			Calc	—	13.55	44.97

The reactions of perchloromethyl mercaptan with aliphatic and aromatic amines (*1–4*), alcoholates, and phenolates (*3, 5*) have been described previously by several investigators. In most cases the products from the reactions were reported to be unstable compounds which decomposed spontaneously on standing or by heating to 130°–140° C. In contrast, the derivatives of perchloromethyl mercaptan prepared in the present investigations have been quite stable under ordinary conditions. The condensation of perchloromethyl mercaptan with imides and amides to form stable compounds containing the *N*-trichloromethylthio group is a general reaction. The four schematic equations (Fig. 1) typify methods by which representatives of different classes of imides and related compounds have been reacted with perchloromethyl mercaptan in the present work.

The > $NSCCl_3$ group, to which the fungicidal activity of the above classes of compounds is attributed, is thus readily introduced into compounds containing an > NH group sufficiently acidic to form a stable alkali metal salt. A general procedure applicable to the preparation of this class of compounds is described below.

One mole of the sodium salt of an imide or amide is dispersed in 1000 ml of benzene in a 2-liter, 3-necked flask equipped with a stirrer, thermometer, reflux condenser, and dropping funnel. While stirring, one mole of perchloromethyl mercaptan is added from the dropping funnel over a period of 2–3 hr. During the addition of mercaptan the reaction mixture is heated slowly from an initial 25° C to refluxing temperature and stirred for an additional 4–6 hr. The benzene solution is filtered hot to remove precipitated NaCl. Isolation of the *N*-trichloromethylthio derivative is then accomplished by evaporating off a portion of the benzene filtrate, cooling, and filtering.

It has also been possible to prepare a closely related product, *N*-trichloromethylthio-morpholine by the direct reaction of perchloromethyl mercaptan with morpholine. An excess of morpholine is employed in this synthesis to tie up the HCl formed in the reaction. *N*-trichloromethylthio-morpholine is a white crystalline material melting at 86°–87° C.

Sixteen compounds which were condensed with perchloromethyl mercaptan are listed in Table 1, along with the empirical formulas and melting points of the new organic compounds produced by the resultant reactions. Biological assays have shown that all the compounds exhibited an LD_{50} value of less than 10 ppm against the fungi *Alternaria solani* and *Sclerotinia fructicola* by the slide germination technique (*6*).[1]

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Manuscript received October 1, 1951.

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[1] The laboratory fungicide tests were carried out in the Standard Oil Development Company's cooperative project at Rutgers University under the supervision of R. H. Daines and Lyle E. Hagmann.

Pre-Cambrian Uraninite, Sunshine Mine, Idaho

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Uranium ore was first discovered in the Sunshine Mine, Coeur d'Alene district, Kellogg, Idaho, in the summer of 1949. Uraninite was subsequently identified as the responsible mineral and occurs in the vicinity of the Sunshine vein at various places from the 2900′ level to the lowest level at 3700′.

The mineral is found in veins somewhat comparable to the nonpegmatitic occurrences of the northwestern Canadian Shield. These veins are cut in a number of places by later siderite veins carrying silver-bearing tetrahedrite. The wall rock belongs to the St. Regis formation (pre-Cambrian quartzite).

Through the geological studies of Ransome and Calkins (*1*), Shenon and McConnel (*2*), and others, the general relationships of the silver veins are well known. The opinion has prevailed (*3*) that the mineral deposits of the Coeur d'Alene are associated with Mesozoic or later igneous activity. Recent reference to the uranium occurrence (*4*) places the uraninite after tetrahedrite, the common silver ore mineral.

Specimens of uranium ores supplied by R. H. Robinson, chief geologist of the Sunshine Mine, studied in the Mineralogical Laboratory of Columbia University, indicate an origin for the uraninite earlier than the tetrahedrite. Laboratory criteria are inadequate to demonstrate, however, whether the uranium was introduced merely at the beginning of the silver-precipitating epoch or during a distinctly earlier mineralization. Arrangements were made, therefore, to secure data for an age determination.

Through the cooperation of the Division of Raw Materials, AEC, both chemical analyses and lead isotope analyses have been secured. The material selected for analysis was collected on the west face of No. 16 stope in the footwall of the Sunshine vein, west of the Jewell crosscut and above the 3100′ level. Uraninite occurs in small veins and segments of veins associated with an alteration halo of fine-grained pyrite and red jasper. Small fragments were broken from vein specimens and examined microscopically for purity. Uraninite was identified by x-ray diffraction, the lattice constant being 5.4439. A chemical analysis of Sunshine uraninite with uraninite analyses from two other localities by Clara Gale Goldbeck, chief, Microchemical Branch, New Brunswick Laboratory, AEC, is given in Table 1. Isotope analyses by Roger Hibbs, chief, Mass Assay Laboratory, Carbide and Carbon Chemicals Company, Oak Ridge, are also included.

On the basis of the analyses, computations have been made by Kulp as given in Table 2 using 4.50×10^9 yr for the half-life of U^{238} and 7.07×10^8 yr for the half-life of U^{235}. Although the half-life of U^{235} has been a subject of debate during the past few years, the value of 7.07×10^8 accepted at present is essentially that proposed by Nier a decade ago.

An uncorrected age from $\left(\frac{Pb}{U + Th}\right)$ of 1050 ± 50 M.Y. is obtained from the graph published by Wick-

TABLE 1

Chemical Analyses

	Sunshine	Martin Lake	Caribou
U_3O_8	26.9	67.5	40.77
Th and R.E.	—	—	1.7 (No Th)
SiO_2	18.2	0.42	
PbO	4.0	7.96	4.15
Al_2O_3	4.4		
Fe_2O_3	—	0.94	
Fe	13.1	—	3.15
CaO	.8	12.7	
MnO	—	0.85	
Mn	1.0		
CuO	—	—	0.55
Cu	7.7	—	
Ag	1.0		
Sb	2.6		
H_2O	1.1	0.62	5.3
CO_2	6.8	4.68	0.22
S	9.	0.01	8.3
V_2O_5		0.3	
NiO			0.34
ZnO			1.22
		Lead Isotopes	
208	17.84 ± 0.07	0.460 ± 0.013	50.67 ± 0.05
207	12.42 ± 0.05	7.32 ± 0.02	20.69 ± 0.03
206	69.20 ± 0.03	92.17 ± 0.01	27.27 ± 0.05
204	0.540 ± 0.010	0.044 ± 0.001	1.37 ± 0.02