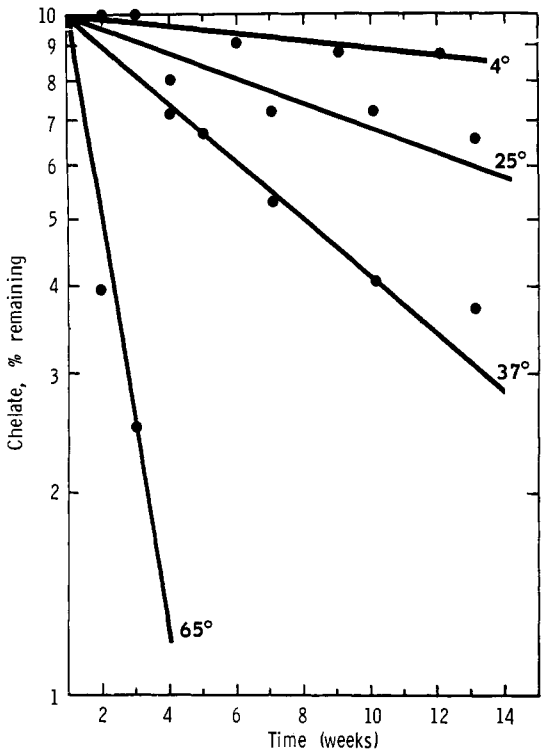

Figure 1.—Per cent chelate $Cu^{II}PTS$ remaining at various temperatures. $Cu^{II}PTS$ was heated at 4, 25, 37, and 65° in air. Samples were chromatographed and radioactivity of products and chelate was determined.

peculiar for this compound alone, but may be a phenomenon associated with all copper chelates. In view of the pharmacological activity shown by a number of chelates,[2,6,9,10] this possible reaction should be kept in mind.

TABLE II

FORMATION OF DECOMPOSITION PRODUCTS OF PTS AND $Cu^{II}PTS$ FOLLOWING HEAT TREATMENT[a]

Time, days	Air I	Air II	Air + MCE I	Air + MCE II	Vac I	Vac II	Vac + MCE I	Vac + MCE II
				$Cu^{II}PTS$				
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	4.3	0.0	5.0	0.0	0.0	0.0	0.0	0.0
1	6.0	15.0	6.5	9.6	2.5	0.0	2.9	0.0
2	10.2	21.0	12.5	18.9	1.0	0.0	3.0	0.0
3	13.0	17.6	14.1	16.4	4.0	0.0	8.0	0.0
5	26.5	15.0	20.6	14.7	6.7	0.0	2.6	0.0
7	39.1		30.5	15.5	7.6	0.0	6.5	0.0
				PTS				
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	3.3	0.0	2.2	0.0	1.8	0.0	1.6	0.0
7	4.5	0.0	4.8	0.0	1.3	0.0	0.7	0.0
10	4.0	0.0	5.2	0.0	2.2	0.0	2.3	0.0

[a] Material was heated at 65°. Values are per cent of starting materials in each product. I is material with R_f 0.09, II with R_f 0.83. The rest of the material was a chelate in sets 1 and 3 and as PTS where MCE was added in sets 2 and 4.

It is possible that the decomposition products are responsible for pharmacological activity of PTS. However, the products found in the urine of treated animals are chromatographically different from the breakdown products *in vitro*.[3] If the products of heating are in fact active intermediates, they are further metabolized *in vivo*. The breakdown products were tested *in vivo*. A sample heated for 9 days at 65° had about 25% of the antitumor effects and 25% as much toxicity as the chelate (Table III). Spectral analysis of the material showed that it contained 22% of unreacted chelate. Thus, the decomposition products are largely inactive.

TABLE III

THE EFFECT OF HEATED AND UNHEATED $Cu^{II}PTS$ ON THE GROWTH OF THE W256 RAT CARCINOSARCOMA[a]

Dose, mg/kg	Unheated AWC, g[b]	Unheated ATD, mm[c]	Unheated Dead	Unheated T/C	Heated AWC, g[b]	Heated ATD, mm[c]	Heated Dead	Heated T/C
0	+35	10.8	0/5	1.00	+35	10.8	0/5	1.00
1.25	+15	9.6	0/5	0.89	+32	10.5	0/5	0.97
2.50	−16	5.5	2/5	0.51	+16	9.6	0/5	0.89
5.00	...	...	5/5	NE[d]	−2	8.4	1/5	0.78
10.0	...	...	5/5	NE[d]	−29	4.5	1/5	0.42

[a] Chelate was prepared as indicated in Experimental Section. The aliquot of chelate to be heated was dissolved in DMF and incubated at 65° for 7 days. The DMF was removed and the material was dried by means of flash evaporation. The compound was suspended in CMC, homogenized, and administered intraperitoneally once daily for 7 days starting 24 hr after implantation. Tumors were evaluated at the end of the therapy period. [b] AWC, average weight change from day of implantation. [c] ATD, average tumor diameter. [d] NE, no evaluation because of animal mortality.

Acknowledgment.—The authors wish to thank Dr. George B. Brown and Dr. C. Chester Stock for their interest in this work and their many helpful suggestions.

New Nitrosamines[1]

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Received October 7, 1967

Numerous N-nitrosoamines have been prepared and studied as carcinogenic agents.[2] We prepared N-nitroso derivatives of amino compounds which had shown either carcinogenic or carcinostatic activity. The yellow, crystalline compounds listed in Table I were prepared by adding the theoretical amount of sodium nitrite to an acid alcoholic solution of the amine at 0–5°, diluting with water, and neutralizing the mixture, then recrystallizing the product from ethanol and from isopropyl ether. Several of them showed marked antitumor effects (see Table I on the following page).

(9) A. C. Sartorelli, A. D. Welch, and B. A. Booth, *Federation Proc.*, **24**, 454 (1965).

(10) E. Mihich, C. L. Simpson, and A. I. Mulhern, *Cancer Res.*, **25**, 1417 (1965).

(1) This investigation was supported in part by Public Health Service Research Grants CA-03717-09-10.

(2) *Cf.* H. Druckrey, R. Preussmann, S. Ivanocic, and D. Schmahl, *Z. Krebsforsch.*, **69**, 103 (1967).

TABLE I

No.	Compound	Yield, %[a]	Mp, °C[b]	Formula[c]	KB cell test, ED_{50},[d] μg/ml	Tumor wt	T/C	Lethality	Killed
						mg/kg		mg/kg	
1	1-(4-N-Ethyl-N-nitrosoamino-benzylidene)indene	53	123–124	$C_{18}H_{18}N_2O$		4×100[e]	0.56	4×400[e]	0/13
						500[f]	0.14		
						1250[f]	0.08	1250[f]	0/3
2	4-(4-N-Ethyl-N-nitroso-aminostyryl)quinoline	59	120.5–122.0	$C_{19}H_{17}N_3O$		4×100[e]	0.10	4×400[e]	1/6
						4×200[e]	0.02		
3	9-(4-N-Methyl-N-nitroso-aminobenzylidene)fluorene	73	145–146	$C_{21}H_{16}N_2O$	100	4×400[e]	0.90	4×400[e]	0/6
						1500[f]	1	1500[f]	0/3
4	1-(4-N-Methyl-N-nitroso-aminobenzylidene)indene	57	144–146	$C_{17}H_{14}N_2O$	35	50[f]	0.15	1500[f]	0/3
						240[f]	0.16		
						600[f]	0.07		
						4×400[e]	0.61	4×400[e]	0/6
5	2-N-Methyl-N-nitrosoamino-fluorene	51	120.5–122.0	$C_{14}H_{12}N_2O$		4×600[e]	0.8	4×600[e]	0/6
6	4-N-Methyl-N-nitrosoamino-stilbene	35	157	$C_{15}H_{14}N_2O$		4×400[e]	0.6	4×400[e]	0/6
7	4-(4-N-Methyl-N-nitroso-aminostyryl)quinoline	84	157–158	$C_{18}H_{15}N_3O$	66	30[f]	0.3	25[f]	1/2
						75[f]	0.15	75[f]	0/3
						4×100[e]	0.45	4×1000[e]	0/6
						4×1000[e]	0.09		

[a] Additional material could be recovered from the mother liquors. [b] Corrected for thermometer stem exposure; determined with Thiele tube. [c] Average of two analyses by Galbraith Laboratories. All compounds were analyzed for C, H. Analytical results obtained for those elements were within ±0.3% of the theoretical values. [d] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at Southern Research Institute and A. D. Little Co. [e] We are grateful to CCNSC for screening tests against Walker 256, using four daily injections beginning 3 days after tumor implant, carried out at Battelle Memorial Institute. [f] We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single intraperitoneal injection in arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts are reported as the ratio T/C.

New Compounds

DL-2-Indaneglycine and DL-β-Trimethylsilylalanine

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Received November 3, 1967

In a study of potential amino acid antagonists, DL-2-indaneglycine and DL-β-trimethylsilylalanine were prepared in order to determine the effect of the fused benzene ring upon the biological activity of known amino acid antagonists, cyclopentaneglycine and cyclopenteneglycine, and to determine whether or not a silicon-containing amino acid might exhibit antimetabolite activity. Neither of the compounds showed any growth-inhibiting properties in several different microorganisms. Steric effects of the large fused ring in the first case and the additional methyl group over that in leucine in the latter case may be responsible for the lack of biological activity of these compounds in binding appropriate enzymes.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected.

2-Bromoindane.—To 2-indanol[1,2] (20 g) in pyridine (3 ml) and 50 ml of $CHCl_3$ at −15° was added PBr_3 (16 ml) over a 30-min period. The reaction mixture was stirred for 2 hr at room temperature and extracted by addition of $CHCl_3$ (100 ml) and ice (100 g). The organic layer was washed with three 50-ml portions of H_2O, dried (Na_2SO_4), and distilled to give 10.9 g of a colorless liquid, bp 83–85° (4 mm), $n^{27}D$ 1.5817. *Anal.* (C_9H_9Br) C, H.

Ethyl α-Acetamido-α-cyano-2-indaneacetate.—To NaOEt, prepared from Na (1.4 g) and EtOH (50 ml), dried *in vacuo*, and suspended in DMSO (50 ml), a solution of ethyl acetamidocyanoacetate (10 g) in DMSO (50 ml) was added with vigorous stirring. 2-Bromoindane (10.5 g) was added dropwise over a 30-min period. The reaction mixture after stirring overnight was concentrated to 25 ml *in vacuo*, diluted with H_2O (100 ml), and extracted three times with 100 ml of Et_2O. The residue from evaporation of the solvent was recrystallized (EtOH–H_2O, then toluene) to yield 6.5 g of white flakes, mp 158–159°. *Anal.* ($C_{16}H_{18}N_2O_3$) C, H, N.

Ethyl α-Acetamido-α-cyano-β-trimethylsilylpropionate.—The above procedure was used to convert 10 g of bromomethyltrimethylsilane (Peninsular ChemResearch, Inc.) to the corresponding derivative of ethyl acetamidocyanoacetate. There was obtained 8.2 g of colorless crystals, mp 99–100°. *Anal.* ($C_{11}H_{20}N_2O_3Si$) C, H, N.

DL-2-Indaneglycine.—A solution of 2.0 g of ethyl α-acetamido-α-cyano-2-indaneacetate and 30 ml of 10% NaOH was refluxed for 12 hr and then acidified to pH 5 with concentrated HCl. A suspension of the resulting precipitate in 500 ml of H_2O was boiled and filtered, and the filtrate after cooling yielded fine crystalline plates. Recrystallization (H_2O) yielded 450 mg of white crystals, mp 323–325° dec. *Anal.* ($C_{11}H_{13}NO_2$) C, H, N. Tlc of this material showed only one purple spot after development with ninhydrin: R_f 0.67 (*n*-BuOH–AcOH–H_2O, 4:1:1), 0.76 (*t*-BuOH–2-butanone–H_2O–28% NH_4OH, 4:3:2:1), 0.85 (H_2O–MeOH, 1:1); pmr absorptions [D_2O, NaOD, 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt internal standard], 2.72

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