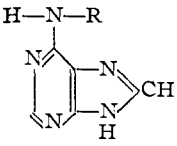
TABLE I

SYNTHESIS OF 6-(SUBSTITUTED)-AMINOPURINES



R	Formula	Wt. 6-methyl mercapto-purine, mg.	Wt. amine, mg.	Time of run,[a] hr.	Yield, %	M.p., °C. dec.[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
2-Furfuryl	$C_{10}H_9N_5O$	1500	3000	12	62	269–270[c]	55.81	56.02	4.22	4.46	32.54	32.98
Benzyl	$C_{12}H_{11}N_5$	200	400	13	66	229	63.98	64.28	4.92	4.77	31.09	31.20
2-Pyridylmethyl	$C_{11}H_{10}N_6$	400	1000	8	45	257	58.39	58.79	4.46	4.12	37.15	37.39
3-Pyridylmethyl	$C_{11}H_{10}N_6$	500	1000	7	58	259	58.39	58.70	4.46	4.43	37.15	37.20
4-Pyridylmethyl	$C_{11}H_{10}N_6$	500	1000	10	50	265–266	58.39	57.82	4.46	4.41	37.15	36.83
2-Thenyl	$C_{10}H_9N_5S$	500	1500	12	52	250	51.93	51.88	3.92	3.89	30.28	30.73

[a] All runs were conducted at a temperature of 130°. [b] Melting points determined on a Fisher melting point block. [c] C. O. Miller, *et al.*, THIS JOURNAL, 77, 2662 (1955), reported m.p. 266–267°.

Some of the biological activities of these compounds are summarized in Table II.[4] At low concentrations (0.01 mg. per l.) not only kinetin but also the benzyl and thenyl derivatives stimulate budding in moss. At higher concentrations (1 mg. per l.) the pyridylmethyl derivatives also stimulate budding, but only the 2-pyridylmethyl compound attains activity comparable to kinetin. At the higher concentration the thenyl derivative becomes inhibitory.

TABLE II

SOME BIOLOGICAL EFFECTS OF 6-(SUBSTITUTED)-AMINOPURINES

Compound	Stimulation of budding of moss[a]	Concn. for 50% inhibition of length of main axis in tomato root cuttings, mg./liter × 10^3
Control	1	..
6-(2-Furfuryl)-aminopurine	20	6
6-Benzylaminopurine	25	6[b]
6-(2-Pyridylmethyl)-aminopurine	1	6[c]
6-(3-Pyridylmethyl)-aminopurine	1	..
6-(4-Pyridylmethyl)-aminopurine	1 (?)	..
6-(2-Thenyl)-aminopurine	20	5[d]

[a] These data were determined at a concentration of 1×10^{-2} mg./liter, and are reported relative to a control equal to *one*. [b] This compound also stimulated the growth in length of the ten basal laterals at 10^{-5} mg./liter. [c] This compound also stimulated the number of laterals at 10^{-4} mg./liter. [d] This compound also stimulated the growth in length of the ten basal laterals at 10^{-6} to 10^{-3} mg./liter.

Preliminary data[5] on the 2-furfuryl, benzyl and 2-thenyl derivatives show an inhibition of growth on the main axis of excised tomato roots (*Lycopersicon esculentum*. Mill.) at concentrations of 5 to 6×10^{-3} mg./liter, whereas the 2-pyridylmethyl derivative is not toxic at this level. Furthermore, the 2-pyridylmethyl derivative increased the growth in length of the main axis at 10^{-4} mg./liter. Also, the benzyl and 2-thenyl compounds stimulate the growth in length of the ten basal laterals, while the 2-pyridylmethyl derivative stimulates the number of laterals at 10^{-5} and 10^{-4} mg./liter, respectively.

From these data, it appears that a number of different 6-(substituted)-aminopurines have potent effects on plant growth.

(4) The authors are indebted to Dr. R. E. Eakin and Mr. B. S. Gorton for the results on moss growth, the details of which will be published separately, and to Dr. W. G. Boll for preliminary data on growth of tomato root cuttings.

(5) W. G. Boll, personal communication.

Experimental

6-Methylmercaptopurine.—The method of Elion and Burge[6] was used except that the course of the reaction appeared somewhat different. Three grams of 6-mercaptopurine[7] was suspended in one equivalent of 2 *N* sodium hydroxide plus 15 ml. of water; and to this well-stirred mixture was added one equivalent of methyl iodide in 9 portions over a period of one hour. The *p*H changed from about 9 to 7, and the mixture set to a semi-solid mass. About 40 ml. of water was then added, and the solid dissolved by warming to yield a solution of *p*H 5, from which 3.4 g. of product separated, m.p. 220° dec.

2-, 3- and 4-Pyridylmethylamines.—These amines were prepared through the catalytic reduction of the corresponding nitrile by the procedure of Kolloff and Hunter.[8]

2-Thenylamine.—This compound was prepared by the method of Hartough[9] through the interaction of thiophene, formaldehyde and ammonium chloride; b.p. 95–99° (28 mm.), $n^{20}D$ 1.5643, reported[9] $n^{20}D$ 1.5615.

6-(Substituted)-aminopurines.—All of these compounds were prepared and recovered in approximately the same manner. One equivalent of 6-methylmercaptopurine and two to three equivalents of the corresponding amine were sealed in a micro Carius tube and heated at about 130° for a given number of hours as noted in Table I. The cooled bomb, in every case, yielded a white to light yellow crystalline mass which was washed with ice-cold ethanol and recrystallized from ethanol or ethanol–water. The final product was dried over P_2O_5 at 120° under vacuum for several hours.

(6) G. B. Elion and E. Burgi, THIS JOURNAL, **74**, 413 (1952).

(7) Burroughs Wellcome and Co., Tuckahoe, N. Y.

(8) H. G. Kolloff and T. H. Hunter, THIS JOURNAL, **63**, 491 (1941).

(9) H. D. Hartough, "Thiophene and Derivatives," Interscience Publishers, Inc., New York, N. Y., 1952, p. 510.

THE BIOCHEMICAL INSTITUTE AND THE
DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TEXAS, AND
THE CLAYTON FOUNDATION FOR RESEARCH
AUSTIN, TEXAS

p-Phenylazophenylsemicarbazones of Trioses and Biologically Related Compounds

BY MAKEPEACE U. TSAO AND ELIZABETH VAN DYKE

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The derivatives of *p*-phenylazophenylsemicarbazide with carbonyl compounds of biological origin are of interest in that they may offer a means of isolation and identification of the latter substances and

may be tested also for possible therapeutic value. It was of particular interest to us to secure a tool for the analysis of trioses. The commonly used 2,4-dinitrophenylosazone, for instance, of dihydroxyacetone and glyceraldehyde are identical; hence, 2,4-dinitrophenylhydrazine is of no value in the separate determination of these trioses, in spite of the fact that the feasibility of colorimetric measurement of the osazones in alkaline solution was generally successful. The *p*-phenylazophenylsemicarbazones were found to form distinct derivatives with dihydroxyacetone, glyceraldehyde and pyruvaldehyde. The solubilities of these derivatives are very different, and mixtures of them can be separated very easily by paper chromatography. For our purposes, however, this result was not yet satisfactory, since no reagent has yet been found which will develop an intense enough color with these derivatives to allow colorimetric determination on a micro-scale.

The synthesis of *p*-phenylazophenylsemicarbazide from the commercially available *p*-aminoazobenzene and the preparation of *p*-phenylazophenylsemicarbazones of simple carbonyl compounds of biological origin are described in this report. For the preparation of *p*-phenylazophenyl isocyanate from *p*-aminophenylazobenzene the procedure of the independent work of Masuyama[1] and Smith[2] was used. The isocyanate was converted into the corresponding semicarbazide by reaction with anhydrous hydrazine. Mixing of an alcoholic solution of the semicarbazide with an aqueous solution of the carbonyl compounds or direct addition to them yields the corresponding semicarbazones at room temperature. Crude semicarbazones are readily purified by recrystallization from ethanol or glacial acetic acid.

Unfortunately, all the *p*-phenylazophenylsemicarbazones prepared in this investigation decompose at melting temperatures; therefore, these derivatives appear to be of little value for the identification of carbonyl compounds by melting point.

Experimental

Melting points are corrected.

p-Phenylazophenylsemicarbazide.—To 100 ml. of stirred dry toluene was added 1 ml. of anhydrous hydrazine, followed by the rapid dropwise addition of a solution of 4.75 g. of crude *p*-phenylazophenyl isocyanate[1,2] in 50 ml. of toluene. The stirring was continued 10 min. after the completion of the addition. The precipitate was collected and washed with toluene, petroleum ether (30–60°) and finally water. The crude material weighed 5.30 g. and was recrystallized from a mixture of pyridine and ethanol. The first two crops of purified product weighed a total of 4.15 g. The over-all yield from *p*-aminoazobenzene hydrochloride to *p*-phenylazophenylsemicarbazide was 76%. The semicarbazide was further purified by recrystallization from ethanol; m.p. 250° dec. Color change in concentrated hydrochloric acid was observed; hence no attempt to prepare the hydrochloride was made.

Anal. Calcd. for $C_{13}H_{13}N_5O$: C, 61.16; H, 5.13; N, 27.44. Found: C, 61.27; H, 5.04; N, 26.86.

Acetaldehyde *p*-Phenylazophenylsemicarbazone.—To a solution of 450 mg. of *p*-phenylazophenylsemicarbazide in 50 ml. of hot 95% ethanol was added 250 mg. of freshly distilled acetaldehyde. On cooling, rosettes of long needles were obtained. The orange crystals were collected and washed with ethanol to yield 420 mg. Ethanol recrystallized material (252 mg.) melted at 231° dec.

Anal. Calcd. for $C_{15}H_{15}N_5O$: C, 64.04; H, 5.37; N, 24.90. Found: C, 64.16; H, 5.14; N, 25.28.

Acetone *p*-Phenylazophenylsemicarbazone.—This compound was prepared in the same manner as the acetaldehyde derivative described above; m.p. 236° dec. Yield of pure product was 345 mg. from 450 mg. of *p*-phenylazophenylsemicarbazide.

Anal. Calcd. for $C_{16}H_{17}N_5O$: C, 65.06; H, 5.80; N, 23.72. Found: C, 65.09; H, 5.97; N, 23.99.

Pyruvic Acid *p*-Phenylazophenylsemicarbazone.—Redistilled crystalline pyruvic acid (130 mg.) was added to a solution of 225 mg. of *p*-phenylazophenylsemicarbazide in hot ethanol. The mixture was allowed to stand overnight and the clear solvent was removed with an air stream. The residue was taken up with minimum amount of hot glacial acetic acid and filtered. On cooling a red orange crystalline product was formed which was collected, washed with ether to yield 270 mg. After recrystallization from glacial acetic acid, it had m.p. 246° dec.; yield of pure product 165 mg.

Anal. Calcd. for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 59.28; H, 4.76; N, 21.21.

α-Ketoglutaric Acid *p*-Phenylazophenylsemicarbazone.—This derivative was prepared in similar manner as that of pyruvic acid described above from 150 mg. of the acid (Sigma Chemical Company); the material was recrystallized from glacial acetic acid to yield 87 mg.; m.p. 233° dec.

Anal. Calcd. for $C_{18}H_{17}N_5O_5$: C, 54.96; H, 4.35; N, 17.81. Found: C, 55.40; H, 4.56; N, 18.22.

The derivatives obtained from pyruvic acid and α-ketoglutaric acid as described above are soluble in dilute alkali.

An attempt to synthesize the *p*-phenylazophenylsemicarbazone of oxalacetic acid yielded a product of m.p. 246° dec. and analysis of C, 58.78; H, 4.61; N, 22.01. This product is very likely the same as that obtained from pyruvic acid; hence decarboxylation seems to have occurred during the recrystallization from hot acetic acid.

Glyceraldehyde *p*-Phenylazophenylsemicarbazone.—One hundred mg. of glyceraldehyde dimer (Sigma Chemical Company) was dissolved in 5 ml. of water and the solution was allowed to stand overnight for conversion into monomer. To this was added a solution of 225 mg. of *p*-phenylazophenylsemicarbazide in 50 ml. of warm ethanol. After standing 10 days only a slight amount of precipitate had formed. The precipitate was removed by filtration and discarded. The filtrate, concentrated to about 3 ml. by air steam and mild heating, was cooled to yield an orange crystalline product. The crude product weighed 220 mg. This material was dissolved in minimum amounts of ethanol and warm benzene was added until just before cloudiness occurs. On cooling a small quantity of yellow crystal was obtained which on exposure to air turned light brown. The filtrate on standing, however, yielded yellow crystals in much larger amounts which were crystallized twice from ethanol–benzene mixture. The stable pure product weighed 58 mg.; m.p. 220° dec.

Anal. Calcd. for $C_{16}H_{17}N_5O_3$: C, 58.70; H, 5.25; N, 21.40. Found: C, 59.03; H, 5.37; N, 19.83.

Dihydroxyacetone *p*-phenylazophenylsemicarbazone was prepared in a similar manner to that for the glyceraldehyde except that 90 mg. of dihydroxyacetone (Dougherty Chemicals) was used and that a flocculent precipitate formed gradually in the reaction mixture. The orange precipitate was collected, washed with ethanol and dried, yield 225 mg. Two recrystallizations from hot ethanol yielded a product weighing 150 mg. with m.p. 220° dec.

Anal. Calcd. for $C_{16}H_{17}N_5O_3$: C, 58.70; H, 5.25; N, 21.40. Found: C, 59.09; H, 5.42; N, 19.90.

Even though the m.p. and the analyses of glyceraldehyde and dihydroxyacetone derivative are identical, the former is very soluble in ethanol while the latter is only moderately soluble. This difference in solubility showed that two distinct compounds had been obtained and that they may be separated with relative ease.

Pyruvaldehyde Bis-*p*-phenylazophenylsemicarbazone.—To a solution of 225 mg. of *p*-phenylazophenylsemicarbazide in 50 ml. of warm ethanol was added 0.3 ml. of a "30% aqueous solution" of pyruvaldehyde (Bios Laboratories).

(1) S. Masuyama and M. Hamada, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **70**, 198 (1949) (*C. A.*, **45**, 6594 (1951)).

(2) P. A. S. Smith, private communication.

The precipitate was collected, washed with ethanol, dried and crystallized twice from glacial acetic acid; m.p. 281° dec. This compound is very slightly soluble in ethanol.

Anal. Calcd. for $C_{29}H_{26}N_{10}O_2$: C, 63.69; H, 4.80; N, 25.63. Found: C, 63.19; H, 4.91; N, 25.71.

Chromatographic Separation of *p*-Phenylazophenylsemicarbazone of Trioses.—All the semicarbazones reported here are very soluble in pyridine hence the latter was useful for the preparation of solutions for chromatographic work. A pyridine solution of a mixture of the semicarbazone of glyceraldehyde, dihydroxyacetone and pyruvaldehyde was applied to a strip of Whatman No. 1 filter paper to make a spot 6–8 mm. in diameter. The paper had been washed with ethanol previously. The paper strip was developed with a methanol:water mixture (2:1), descending technique was employed. When the solvent front had traveled 40 cm. or more three distinct spots were observed. The pyruvaldehyde derivative remained at the origin as in the case when it was developed as a single substance. The glyceraldehyde derivative has moved further down than the dihydroxyacetone derivative. When developed individually these two derivatives have the following R_f values (27°): glyceraldehyde derivative, 0.61; dehydroxyacetone, 0.52.

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DEPARTMENT OF PEDIATRICS AND
COMMUNICABLE DISEASES
UNIVERSITY OF MICHIGAN MEDICAL SCHOOL
ANN ARBOR, MICHIGAN

Thiourea Adduct of 1,2,4,5-Tetramethylbenzene

BY J. W. TETER AND W. P. HETTINGER, JR.

RECEIVED JULY 15, 1955

Although benzene itself and most alkyl benzenes do not form adducts with thiourea, we find that durene readily forms a stable adduct. X-ray diffraction patterns of the solid obtained in adduction experiments show the presence of some thiourea but also clearly indicate adduct formation.

We have used this adduct as a means of isolating durene from fractions of high-octane-reforming products boiling in the neighborhood of 196°, the durene content of which can be estimated by cryoscopic means.

The fact that only durene adduction was observed in the presence of other tetramethylbenzene isomers indicates the selectivity of the reaction. Likewise none of the C_8-, C_9- or C_{11}-methyl substituted benzenes appeared to form adducts approaching the stability of durene.

Experimental

A solution of dry methanol, saturated with thiourea at 25° (150 ml.), was mixed with 26.6 ml. of a solution containing 21.9 volume per cent. durene and 78.1 volume per cent. toluene. Almost immediately a voluminous precipitate of white needles was formed. This solution plus precipitate was then cooled to 3°. The remaining mother liquor contained 91.6% toluene and 8.4% durene on a methanol and thiourea free basis.

The precipitate was collected, dried and weighed (12.9 g.) and analyzed.

Anal. Found: C, 37.2; H, 6.3; N, 26.2; S, 30.3.

X-Ray Examination.—Samples of durene, thiourea and the solid complex were analyzed on a North American Phillips X-ray spectrometer employing CuKα radiation. Table I shows the results of these analyses in terms of *d/n*.

TABLE I

X-RAY DIFFRACTION PATTERNS

Durene thiourea add.		Durene		Thiourea	
d/n	Int.	*d/n*	Int.	*d/n*	Int.
7.61	10	....	..	....	..
....	..	6.44	100	....	..
5.80	20	....	..	....	..
....	..	5.29	14	....	..
....	..	5.20	24	....	..
....	..	5.02	10	....	..
4.49	15	....	..	4.60	10
4.438	100	....	..	4.435	100
....	..	4.38	5	....	..
4.308	50	....	..	4.252	100
....	..	3.89	15	....	..
3.85	4	3.85	15	3.82	80
3.64	6	....	..	....	..
3.51	50	....	..	3.50	70
3.37	40	....	..	....	..
....	..	3.22	20	....	..
....	..	....	..	3.13	100
2.93	5	....	..	3.08	70

The data show that a new crystalline substance has been formed. Similarity of the cyclohexane and durene adduct patterns further confirms this.

Acknowledgment.—The authors wish to express their appreciation to Sinclair Research Laboratories for permission to publish this information. They also wish to acknowledge the assistance of Mr. R. A. Van Nordstrand who contributed the X-ray data presented here.

CATALYSIS RESEARCH DIVISION
SINCLAIR RESEARCH LABORATORIES, INC.
HARVEY, ILLINOIS

4-Methyl-4-(3,5-Dimethylphenyl)-2-Methylpentene-1. An Intermediate Compound in the Synthesis of 1,1,3,3,4,6-Hexamethylindan

BY PHILIPPE TEYSSIÉ AND GEORGES SMETS

RECEIVED OCTOBER 10, 1955

During the course of an investigation on intramolecular cyclization in polymeric systems,[1] the synthesis of several reference substances has been carried out in order to allow an infrared spectrometric determination of the structure of this new class of polymers. For this purpose, 1,1,3,3,4,6-hexamethylindan has been prepared according to the method of Smith and Spillane[2]: reaction of methyl (3,5-dimethylphenyl)-isovalerate (I) with methylmagnesium iodide, and subsequent dehydration of the crude carbinol II in the presence of sulfuric acid. Owing to its insensitivity to ozone and alkaline permanganate in the cold, a structure of 1,1,3,3,4,6-hexamethylindan (IV) was assigned[2] to the hydrocarbon obtained. However, the same procedure in our laboratory gave a hydrocarbon different from that described by Smith and Spillane; it has been proved to be 4-methyl-4-(3,5-dimethylphenyl)-2-methylpentene-1 (III). Moreover, in the presence of aluminum chloride, internal cyclization occurs and the pentene III is trans-

(1) Ph. Teyssié and G. Smets, Friedel–Crafts Reactions on Polyvinyl Chloride, *J. Polymer Sci.*, in press.

(2) L. I. Smith and J. L. Spillane, THIS JOURNAL, **65**, 202 (1943).