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The Preparation of Acylated Derivatives of 2-Amino-6-purinethiol and Related Compounds

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The preparation and properties of N-acylated derivatives of guanine, 2-amino-6-purinethiol and their related compounds were described.

The position of the N-acyl group introduced was deduced to be the 2-amino group of purine ring from the elemental analysis and the comparison of ultraviolet spectra of related compounds.

During the acylation procedure, desulfurization of 2-amino-6-purinethiol and alkyl substituted compound was observed.

Quite a number of derivatives of S-substituted[2-4] and 9-substituted[5] 2-amino-6-purinethiols have so far been synthesized to investigate the relation between the chemical structures and their anti-tumor activities with the consequence of interesting findings in the biological activities of 9-substituted 2-amino-6-purinethiol.[5]

Recently, Sartorelli and Le Page[6] have shown that 2-amino-6-purinethiol reveals its anti-tumor effect by inhibiting the biosynthesis of nucleic acid at several sites. It is expected, therefore, that, by modifying the chemical structure of 2-amino-6-purinethiol, anti-tumor agents with high specificity and with less toxicity can be obtained which may have affinity at fewer enzyme sites.

The above supposition is confirmed by the fact that 2-amino-9-β-D-ribofuranosyl-6-purinethiol[7] and 2-amino-9-*n*-propyl-6-purinethiol[5] exert their anti-tumor effect against Adenocarcinoma 755 with better therapeutic index. Furthermore, a number of pyrimidine and azapyrimidine nucleosides,[8] purine nucleosides[7,9] and nucleoside antibiotics[10] have been acylated to investigate with respect to the biological activity, and it is reported that these acyl derivatives show quite different absorption patterns when administered per oral,[8,10] owing to their greater lipophylic properties. The effect of acylation on these nucleosides can be interpreted that the permeability of these anti-tumor agents through the cell membrane is altered thus the absorption and transportation of the anti-tumor agents are changed.

One of the major problems in the treatment of tumor by 6-mercaptopurine and the related derivatives is that these compounds are rapidly metabolized, transformed into the

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2) G.B. Elion, I. Goodman, W. Lange, and G.H. Hitchings, *J. Am. Chem. Soc.*, **81**, 1898 (1959).
3) D.A. Clarke, G.B. Elion, G.H. Hitchings, and C.C. Stock, *Cancer Res.*, **18**, 445 (1958).
4) G.D. Daves, Jr., C.W. Noell, R.K. Robins, H.C. Koppel, and A.G. Beaman, *J. Am. Chem. Soc.*, **82**, 2633 (1960).
5) C.W. Noell and R.K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).
6) A.C. Sartorelli and G.A. LePage, *Cancer Res.*, **18**, 1329 (1958).
7) J.J. Fox, I. Wempen, A. Hampton, and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).
8) *a*) E.S. Perkins, R.M. Wood, M.L. Sears, W.H. Prusoff, and A.D. Welch, *Nature*, **194**, 985 (1962); *b*) K.L. Mukherjee and C. Heidelberger, *Cancer Res.*, **22**, 815 (1962); *c*) W.A. Creasey, M.E. Fink, R.E. Handschmacher, and P. Calabresi, *ibid.*, **23**, 444 (1963).
9) *a*) J.F. Gerster, A.G. Beaman, and R.K. Robins, *J. Med. Chem.*, **6**, 340 (1963); *b*) J.F. Gerster, J.W. Jones, and R.K. Robins, *J. Org. Chem.*, **28**, 945 (1963).
10) H. Hoeksema, G.B. Whitfield, and L.E. Rhuland, *Biochem. Biophys. Res. Commun.*, **6**, 213 (1961).

inactive form and easily excreted.[11] One of the ways to avoid the rapid inactivation and to keep the efficiency of these anti-tumor agents as long as possible is to modify the compounds into the masked derivatives which will gradually be converted *in vivo* into the desired active form.

In accordance with the above mentioned viewpoint, the authors have attempted to synthesize the derivatives of 2-amino-6-purinethiol and alkyl substituted 2-amino-6-purinethiol acylated at 2-amino group in the purine ring for the purpose of comparing the 2-acylamido derivatives with the parent compounds in their anti-tumor activity, toxicity and other properties. This paper deals with the part of the preparation of N-acylated derivatives of 2-amino-6-purinethiol and its related compounds.

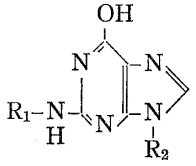
Experiments for biological activities of these N-acylated compounds on various experimental tumors were performed at National Cancer Center Research Institute and preriminarily reported at the 22nd, 23rd and 24th Annual Meeting of Cancer Society of Japan. The comprehensive report about these experiments will be published within soon.

There are few report on the N-acylation of guanine and/or 9-substituted guanine, while full acetylation of deoxyguanosine[12] and its 5'-phosphate[13] had been reported. Bowles, *et al.*[14] reported the synthesis of 2-acetamido-6-benzyloxypurine by acetylating 2-amino-6-benzyloxypurine in acetic anhydride/toluene under refluxing.

After various trials by applying conventional N-acylation methods, acid anhydride of corresponding acid was chosen as acylating agent as well as the solvent, as all the compounds we treated gave products with only one acyl group (listed in Table I, II and III).

The position of the one acyl group introduced into guanine, 2-amino-6-purinethiol and their alkyl substituted analogs is not yet fully elucidated, however the following reasons lead the authors to believe that the acyl group was introduced on the 2-amino group of purine ring.

Table I. Ultraviolet Spectra of N-Acylated Derivatives of Guanine and 9-Alkyl Substituted Guanines



Compound	R_1	R_2	λ_{max} mμ (ε) pH 1	pH 6	pH 12
N-Isobutyrylguanine (I)	$(CH_3)_2CHCO$	H	265 (15, 150)	268 (15, 510)	276 (10, 000)
N-Isobutyryl-9-methylguanine (II)	$(CH_3)_2CHCO$	CH_3	263 (15, 800)	260 (14, 500) 280[a]	265 (11, 250)
N-Isobutyryl-9-*n*-propylguanine (III)	$(CH_3)_2CHCO$	*n*-C_3H_7	265 (15, 470)	260 (15, 000) 280[a]	263 (10, 340)
N-Formyl-9-*n*-propylguanine (IV)	HCO	*n*-C_3H_7	260 (12, 150)	261 (10, 570) 280[a]	263 (8, 490)

a) inflection

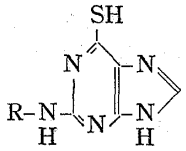
11) a) G.B. Elion, S. Bieber, and G.H. Hitchings, *Ann. N.Y. Acad. Sci.*, **60**, 297 (1954); b) E.J. Sarcione and L. Stutzman, *Cancer Res.*, **20**, 387 (1960); c) G.B. Elion, S. Callahan, and G.H. Hitchings, *Proc. Am. Assoc. Cancer Res.*, **3**, 316 (1962); d) H.J. Hansen, W.G. Giles, and S.B. Nadler, *Proc. Soc. Exptl. Biol. Med.*, **113**, 163 (1963); e) G.B. Elion, S. Callahan, H. Nathan, S. Bieber, R.W. Rundles, and G.H. Hitchings, *Biochem. Pharmacol.*, **12**, 85 (1963).

12) H. Schaller, G. Weimann, B. Lerch, and H.G. Khorana, *J. Am. Chem. Soc.*, **85**, 3821 (1963).

13) R.K. Ralph, W.J. Connors, H. Schaller, and H.G. Khorana, *J. Am. Chem. Soc.*, **85**, 1983 (1963).

14) W.A. Bowles, F.H. Schneider, L.R. Lewis, and R.K. Robins, *J. Med. Chem.*, **6**, 471 (1963).

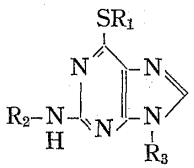
Table II. Ultraviolet Absorption Spectra of 2-Acylamido-6-purinethiols



Compound	R	λ_{max} mμ (ε) pH 1	pH 6	pH 11
2-Formamido-6-purinethiol (V)	HCO		333 (15, 000) 248[a]	253 (16, 650) 320 (16, 100)
2-Acetamido-6-purinethiol (VI)	CH_3CO	337 (15, 110) 240,[a] 285[a]	335 (16, 450) 290[a]	248 (16, 150)[b] 320 (14, 520)
2-Isobutyramido-6-purinethiol (VII)	$(CH_3)_2CHCO$	337 (15, 670) 242,[a] 285[a]	336 (17, 030) 288[a]	251 (17, 360) 316 (19, 250)
2-Capryloylamido-6-purinethiol (VIII)	$CH_3(CH_2)_6CO$	339 (15, 100) 242,[a] 286[a]	341 (14, 600)[c] 295[a]	254 (15, 600) 320 (14, 700)

a) inflection b) measured at pH 12 c) measured in 95% EtOH

Table III. Ultraviolet Absorption Spectra of N^2-Acylated Derivatives of Alkyl Substituted 2-Acylamido-6-purinethiols



Compound	R_1	R_2	R_3	λ_{max} mμ (ε) pH 1	pH 6	pH 11
2-Isobutyramido-6-methylthiopurine (IX)	CH_3	$(CH_3)_2CHCO$	H	250 (17, 100) 305 (12, 970) 275[a]	247 (22, 400) 296 (13, 550) 225[a]	243 (27, 000) 300 (15, 650)
2-Isobutyramido-9-methyl-6-purinethiol (X)	H	$(CH_3)_2CHCO$	CH_3	337 (18, 980) 245,[a] 298[a]	333 (20, 670) 297[a]	255 (21, 100) 315 (21, 560)
2-Isobutyramido-9-*n*-propyl-6-purinethiol (XI)	H	$(CH_3)_2CHCO$	n-C_3H_7	338 (18, 320) 245,[a] 290[a]	335 (20, 800) 294[a]	255 (17, 690)[b] 315 (19, 780)
2-Formamido-9-*n*-propyl-6-purinethiol (XII)	H	HCO	n-C_3H_7	340 (20, 480) 245[a]	327 (21, 430)	250 (14, 070)[b] 320 (20, 280) 270[a]
2-Isobutyramido-9-methyl-6-methylthiopurine (XIII)	CH_3	$(CH_3)_2CHCO$	CH_3	252 (19, 200) 306 (12, 950)	248 (22, 840) 297 (16, 330)	248 (23, 700) 295 (16, 200)

a) inflection b) measured at pH 12

i) The ultraviolet (UV) absorption spectra of isobutyrilated 9-methylguanine and 9-*n*-propylguanine is similar to that of N^2-acetyl-9-benzylguanine[15] which was synthesized by another method.

ii) Isobutyrilated 2-amino-6-methylthiopurine (IX) and 2-amino-9-methyl-6-methylthiopurine (XIII) gave UV spectra similar to 2-acetamido-6-benzylthiopurine and 2-acetamido-6-benzylthio-9-(tetrahydro-2-furyl)purine.[14]

15) B. Shimizu and M. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **15**, 1066 (1967).

iii) The treatment of mono–isobutyrilated guanine and 2–amino–6–purinethiol with mild alkaline did ont affect the acylated products. The above fact suggests that the acylation did not occur at N^7 nor at N^9.[16]

iv) The possibility of acylation of S^6 and N^1 was abolished when investigating the UV spectra of acylated 2–amino–6–purinethiol at various pH values. When pH of the media was changed, the change of ionization at S^6 and N^1 was clearly revealed.

During the acylation procedure, desulfurization was observed on 2–amino–6–purinethiol and its alkyl substituted analog. For instance, when 2–amino–9–methyl–6–purinethiol was acylated in isobutyric anhydride at rather drastic condition, N^2–isobutyryl–9–methylguanine was formed as the result of the desulfurization. If N^2–isobutyramido–6–purinethiol was subjected to the similar condition, the formation of N^2–isobutyrylguanine is possible to confirm.

Experimental

N-Isobutyrylguanine (I)——One gram of dry guanine was refluxed with freshly distilled isobutyric andryhide for 6 hr. Initially, guanine did not dissolve into the anhydride even at the boiling point, however, by prolonged reaction, guanine seemed to dissolved in very gradually with slight yellow–coloring. Although guanine did not dissolve away in the anhydride after 6 hr heating, the shape of the crystal in the reaction flask changed from amorphous, powdery crystal to fibrous, long crystal. The crystalline precipitates were collected and recrystallized from abs. ethanol. The yield was 385 mg, and 414 mg of guanine was recovered. The melting point of the product was above 285°. *Anal.* Calcd. for $C_9H_{11}O_2N_5$ (N^2–isobutyrylguanine): C, 48.90; H, 5.01; N, 31.69. Found: C, 48.72; H, 4.80; N, 32.06.

N-Isobutyryl-9-methylguanine (II)——Two hundred and fifty milligrams of 9–methylguanine[5] was refluxed with 6.5 g of isobutyric anhydride for 4 hr. The suspension turned to a clear brown solution, which was well cooled in a refrigerator after the reaction time. Precipitated crystal was collected on a glass filter, washed with ether, and recrystallized from abs. ethanol using active charcoal to yield 228 mg of colorless plate, mp 277—278.5°. *Anal.* Calcd. for $C_{10}H_{13}O_2N_5$ (N^2–isobutyryl–9–methylguanine): C, 51.05; H, 5.57; N, 29.77. Found: C, 50.88; H, 5.49; N, 30.21.

N-Isobutyryl-9-*n*-propylguanine (III)——Fifty milligrams of 9–*n*–propylguanine[5] was heated with 5 ml of isobutyric anhydride at 140° for 1 hr and refluxed for 2 hr. Exess of isobutyric anhydride was evaporated under reduced pressure at 100° and the residual crystalline mass was treated with abs. ether. The crystal was collected on a filter and recrystallized from ethanolic water to yield colorless needles, mp 218—219° (48 mg). *Anal.* Calcd. for $C_{12}H_{17}O_2N_5$ (N^2–isobutyryl–9–*n*–propylguanine): C, 54.73; H, 6.50; N, 26.60. Found: C, 54.52; H, 6.41; N, 27.14.

N-Formyl-9-*n*-propylguanine (IV)——Thirty milliliters of acetic anhydride and 15 ml of formic acid (100%) were mixed in a flask under ice–cooling and the mixture was warmed at 50—60° for 2 hr. In this procedure, formic acetic anhydride is formed.[17] A mixture of the acetic formic anhydride solution and 0.5 g of 9–*n*–propylguanine was heated at 95—100° for 2 hr with vigorous stirring. After the reaction time, the excess anhydride was evaporated to dryness under reduced pressure at 40°. After treatment with ether, crystal mass was separated and recrystallized from abs. methanol to afford 350 mg of micro sand like crystals, which had a mp of 313° (decomp.). *Anal.* Calcd. for $C_9H_{11}O_2N_5$ (N^2–formyl–9–*n*–propylguanine): C, 48.86; H, 5.02; N, 31.66. Found: C, 48.81; H, 5.15; N, 31.79.

2-Formamido-6-purinethiol (V)——One gram of 2–amino–6–purinethiol was suspended in 200 ml of formic acetic anhydride and the reaction mixture was heated to 110—120° for refluxing with vigorous stirring. After 2 hours, the reaction mixture was evaporated to dryness under reduced pressure to remain yellow powder, which was washed twice with hot ethanol. The slightly colored powder was recrystallized from water to afford 470 mg of thin–yellowish powder. The melting point of the product was above 300°. *Anal.* Calcd. for $C_6H_5ON_5S$ (2–formamido–6–purinethiol): C, 36.85; H, 2.56; N, 35.87; S, 16.42. Found: C, 36.51; H, 3.60; N, 35.91; S, 16.49.

2-Acetamido-6-purinethiol (VI)——In a mixture of 70 ml of acetic anhydride and 180 ml of freshly distilled pyridine, 3 g of finely powdered 2–amino–6–purinethiol was suspended, stirred at room temperature for 3 hr and at 23° for 16 hr. After the reaction time, most of pyridine was evaporated under reduced pressure at 40° and the residue was treated with 140 ml of abs. ethanol to precipitate. After the separation of the precipitate (500 mg), the filtrate was evaporated to a half volume of the initial one and cooled in a refrigerator

16) J.A. Montgomery, *J. Am. Chem. Soc.*, **78**, 1928 (1956).
17) *a*) C.W. Huffman, *J. Org. Chem.*, **23**, 727 (1958); *b*) K. Freudenberg, *Chem. Ber.*, **80**, 325 (1947).

overnight to separate second precipitate (800 mg). Two lots of the precipitate were combined and recrystallized from hot water repeatedly to afford 1.27 g of a pure product. The melting point of the product was above 280°. *Anal.* Calcd. for $C_7H_7ON_5S \cdot H_2O$ (2–acetamido–6–purinethiol): C, 37.01; H, 3.99; N, 30.83; S, 14.10. Found: C, 37.52; H, 4.25; N, 31.23; S, 14.33.

2-Isobutyramido-6-purinethiol (VII)——Two grams of dried 2–amino–6–purinethiol was suspended in 35 g of isobutyric anhydride, which was heated at 145—150° under stirring for 5 hr. After the reaction time, the reaction mixture was cooled and the precipitate was taken up on a glass filter, which was washed with cold ethanol and ether. Recrystallization from hot ethanol yielded thin yellowish crystals showing a mp 245°. Additional 370 mg of the product was recovered from mother liquor. *Anal.* Calcd. for $C_9H_{11}ON_5S$ (2–isobutyramido–6–purinethiol): C, 45.56; H, 4.68; N, 29.53; S, 13.46. Found: C, 45.28; H, 4.96; N, 28.84; S, 13.82.

2-Capryloylamido-6-purinethiol (VIII)——A mixture of 3.5 g of dry 2–amino–6–purinethiol and 55 ml of capryloyl anhydride (bp 160—163° (6 mmHg)) was heated at 100° for 2 hr, and at 150° for 2 hr with stirring. After the reaction time, the mixture was filtered on a glass filter while hot, and the precipitate was washed with hot ethanol. The filtrate and ethanol washings were combined and evaporated to dryness at reduced pressure. The residue was dissolved in abs. ethanol and concentrated to a small volume. From the concentrates, the precipitate was collected and recrystallized twice from 300 ml of ethanol to yield 550 mg of fine crystals. mp 240°. *Anal.* Calcd. for $C_{13}H_{19}ON_5S$ (2–capryloylamido–6–purinethiol): C, 53.23; H, 6.53; N, 23.88; S, 10.93. Found: C, 54.29; H, 6.56; N, 23.80; S, 11.14.

2-Isobutyramido-6-methylthiopurine (IX)——A mixture of 1.2 g of 2–amino–6–methylthiopurine[18] and 25 g of isobutyric anhydride was heated at 150° for 2 hr. After the reaction time, the mixture was filtered while hot, and isobutyric anhydride was evaporated under reduced pressure at 100°. Residual material was treated with abs. ethanol and re–evaporated to yield powdery product, which was recrystallized from hot ethanol using active charcoal. Colorless fine needle was isolated, which had a mp of 251—252° (decomp.). Yield, 844 mg. *Anal.* Calcd. for $C_{10}H_{13}ON_5S$ (2–isobutyramido–6–methylthiopurine): C, 47.80; H, 5.22; N, 27.88; S, 12.76. Found: C, 48.02; H, 5.27; N, 27.63; S, 12.91.

2-Isobutyramido-9-methyl-6-purinethiol (X)——A mixture of 1.4 g of 9–methyl–6–thioguanine[19] and 150 g of isobutyric anhydride was heated at 140—145° for 3 hr with vigorous stirring. After the reaction time, excess isobutyric anhydride was distilled off at reduced pressure to remain brown–colored powder, which was extracted 3 times with 150 ml portions of abs. ethanol. Resulting extract, which had reddish–brown color, was treated with active charcoal and evaporated to small volume. Recrystallization from ethanol yielded 360 mg of yellow colored needles, which had a mp of 222—223°. *Anal.* Calcd. for $C_{10}H_{13}ON_5S$ (2–Isobutyramido–9–methyl–6–purinethiol): C, 47.80; H, 5.22; N, 27.88; S, 12.76. Found: C, 46.74; H, 5.29; N, 27.22; S, 12.88.

2-isobutyramido-9-*n*-propyl-6-purinethiol (XI)——A mixture of 0.75 g of 2–amino–9–*n*–propyl–6–purinethiol[5] and 20 ml of isobutyric anhydride was heated at 135—140° for 2 hr with stirring. After the reaction time, excess isobutyric anhydride was distilled off at reduced pressure, twice. Resulting clear borwn resin was treated with ether to afford grayish powder (0.7 g), which was collected on a filter. Recrystallization from water containing dioxane using active charcoal yielded 350 mg of colorless needles, which had a mp of 213°. *Anal.* Calcd. for $C_{12}H_{18}ON_5S$ (2–isobutyramido–9–*n*–propyl–6–purinethiol): C, 51.58; H, 6.49; N, 25.10; S, 11.48. Found: C, 51.90; H, 6.52; N, 25.67; S, 11.07.

2-Formamido-9-*n*-propyl-6-purinethiol (XII)——Formic acetic anhydride was prepared from 39 ml of acetic anhydride and 19.5 ml of abs. formic acid as previously described. A mixture of formic acetic anhydride above prepared and 0.6 g of 2–amino–9–*n*–propyl–6–purinethiol was heated at 105—110° for 1.5 hr with vigorous stirring. Most of the material had dissolved after this reaction time and the reaction mixture was filtered while hot. After standing overnight in a refrigerator, white crystal appeared, which was filtered on a glass filter. Recrystallization from abs. methanol yielded 480 mg of colorless fine needles, which had mp 259° (decomp.). *Anal.* Calcd. for $C_9H_{11}ON_5S$ (2–formamido–9–*n*–propyl–6–purinethiol): C, 45.61; H, 4.67; N, 29.52; S, 13.52. Found: C, 46.02; H, 4.79; N, 30.14; S, 13.74.

2-Isobutyramido-9-methyl-6-methylthiopurine (XIII)——Eight hundreds milligrams of 2–amino–9–methyl–6–methylthiopurine[18] was suspended in 9 g of isobutyric anhydride and heated at 150° for 1.5 hr. After the reaction, excess isobutyric anhydride was evaporated under reduced pressure below 100° and the residue was added with abs. ethanol, re–evaporated under reduced pressure. The resultant brownish sirup was extracted with hot ethylacetate and the extract was decolorized with active charcoal, concentrated to dryness. Recrystallization from ethylacetate yielded 420 mg of needles, showing mp 186—187°. *Anal.* Calcd. for $C_{11}H_{15}ON_5S$ (2–isobutyramido–9–methyl–6–methylthiopurine): C, 49.80; H, 5.70; N, 26.40; S, 12.08. Found: C, 49.36; H, 5.74; N, 26.29; S, 12.31.

Conversion of 2-Isobutyramido-6-purinethiol (VII) to N-Isobutyrylguanine (I)——2–Isobutyramido–6–purinethiol (350 mg) was suspended in 55 g of isobutyric anhydride and heated at 160—170°. Gradually,

18) E.O. Leonard, C.G. Skinner, E.M. Lansford, and Jr., W. Shive, *J. Am. Chem. Soc.*, **81**, 907 (1959).
19) H.C. Koppel and R.K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).

(VII) dissolved away in the anhydride and the solution turned to yellow to brown–red color. After 8 hr, the reaction mixture was evaporated under reduced pressure to a sirup which was recrystallized from abs. ethanol after treatment of active charcoal. Yield, 180 mg. From the data of the UV spectrum and the elemental analysis, this product was identified with N^2–isobutyryl guanine. *Anal.* Calcd. for $C_9H_{11}O_2N_5$ (N–isobutyryl guanine): C, 48.90; H, 5.01; N, 31.69. Found: C, 49.03; H, 4.87; N, 31.85. UV λ_{max}^{PH1} 265, λ_{max}^{PH6} 268, λ_{max}^{PH12} 276 mμ.

Conversion of 2-Amino-9-methyl-6-purinethiol to N-Isobutyryl-9-methylguanine (II)——2–Amino–9–methyl–6–purinethiol (1 g) was suspended in 20 ml of isobutyric anhydride and heated at 145—150° for 3½ hr and refluxed at 220° for additional 2 hr. Most of the material had dissolved after this reaction time, and the reaction mixture was filtered while hot. After standing overnight in a refrigerator, white crystal appeared, which was recrystallized repeatedly from abs. ethanol to yield colorless plate crystal (380 mg). This product had a mp of 275—277°, undepressed by admixture with authentic N–isobutyryl–9–methylguanine, mp 277—278.5°. *Anal.* Calcd. for $C_{10}H_{13}O_2N_5$ (N–isobutyryl–9–methylguanine): C, 51.05; H, 5.57; N, 29.77. Found: C, 51.07; H, 5.35; N, 30.01. UV λ_{max}^{PH1} 263, λ_{max}^{PH6} 260, 280 (Inflection), λ_{max}^{PH12} 265 mμ.

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