phenone le. Several recrystallizations from acetone provided a pure specimen as colorless plates, m.p. 110–110.5°.

Anal. Calcd. for $C_{11}H_{20}Cl_3NO$: C, 45.77; H, 6.99; Cl, 36.85. Found: C, 45.32; H, 7.07; Cl, 36.79.

6-Acylamido- and 6-Acylamido-9(or 7)-acylpurines[1]

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Both 6-acylamido-9(or 7)-acylpurines and 6-acylamidopurines were synthesized. Various chemical, spectral and chromatographic characteristics of the above compounds are described. Neither rat liver cell fractions, homogenates of mammalian organs nor proteolytic enzymes hydrolyze 6-acylamidopurines. Anticancer screening yielded negative results.

Compounds such as 6-chloropurine[2] and 6-mercaptopurine[2,3] have pronounced effects on cell division. 6-Methylpurine alters cellular enzyme levels.[4] Other compounds in which the amino group of adenine is alkyl or aryl substituted, *e.g.*, puromycin[5,6] and kinetin, inhibit protein synthesis and cell growth.[2] The pharmacological action of puromycin has been associated with the alkyl substituted amino group; substitution of the 6-dimethylaminopurine moiety by adenine resulted in a loss of biological effect.[7] An analog of kinetin, 6-N-β-indolylethyladenine causes a disturbance of mitosis in normal and malignant cells and lyses tissue cultures of sarcomas and car-

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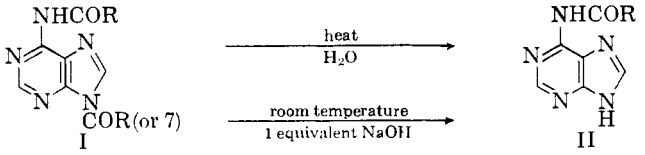
cinomas.[8] 6-Methylaminopurine and 6-dimethylaminopurine have been found in RNA of bacteria, yeast, and rat liver microsomes.[9]

We are reporting the synthesis and properties of several 6-acylamidopurines, hereafter referred to as diacyl (I) and monoacyl (II) adenines or fatty acid amides of adenine. Monoacyladenines of some



I II

R = $-CH_3$, $-C_2H_5$, $-(CH_2)_2CH_3$, $-(CH_2)_3CH_3$, $-(CH_2)_4CH_3$, $-(CH_2)_5CH_3$

longer chain fatty acids C_6–C_{18} have been prepared by heating the respective acid chlorides with adenine in pyridine. It is claimed that these derivatives of adenine are potent virus inhibitors.[10] Some fatty acid amides of adenine are described for the first time. Suitable quantities have been prepared for testing by the Cancer Chemotherapy National Service Center; results of enzymatic and metabolic investigation to date will also be reported.

Results and Discussion.—Diacyl (I) and monoacyladenines (II) are obtained as the direct result of a reaction between adenine and an acid anhydride. Thus, Birkofer obtained only monopropionyl and monobutyryladenine, but the reaction with acetic anhydride yielded diacetyladenine.[11] However, Kossel[12] and Davoll and Lowy[13] isolated only monoacetyladenine by refluxing adenine with acetic anhydride. In our hands adenine and acetic anhydride yielded only diacetyladenine. Recrystallization of this compound from boiling water causes hydrolysis of one amide bond to monoacetyladenine.[11]

The procedure employed by us yielded diacyladenines in all cases. While it is possible that small amounts of monoacyladenines were present in the reaction mixture, they would have been eliminated by virtue of their insolubility in hot toluene. The lability of one of the acetyl groups of diacetyladenine has been related to its position on a

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(12) A. Kossel, *Z. physiol. Chem.*, **12**, 241 (1888).

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particular ring nitrogen. Montgomery[14] employed warm 10% sodium hydroxide solution to remove purine 9-(or 7-) "active N-acetyl." We have observed that this procedure may also hydrolyze the 6-acylamidopurine bond. Baker and Hewson[15] refluxed 2,6-diaminopurine with acetic anhydride and obtained 2,6-diacetamido-9(or 7)-acetylpurine. Recrystallization from hot water hydrolyzed the 9(or 7) acetyl group yielding 2,6-diacetamidopurine. Analogously it seems likely that one acyl is substituted on N9 (or 7) in the diacyladenine series since diacyladenines are readily convertible to monoacyladenines by heating or by sodium hydroxide below pH 11.

The values for C, H, N, and particularly oxygen (determined directly, Table I, dibutyryladenine) support a diacyl rather than a monoacyl salt structure for the diacyladenines.

Preliminary studies revealed that the 6-acylamidopurine bond was labile to mild alkaline hydrolysis. Since enzymatic and metabolic studies with acyladenines were contemplated, it was necessary to determine the stability of these compounds at various temperatures and conditions of pH. The monoacyladenines were essentially stable at room temperature, pH 3.3-8.1 and were partially hydrolyzed on both sides of this pH range. At 50° for 7 hours, the stability range was essentially pH 3.3–7.1. At 80° the compounds were stable only at pH 5.1. At 100° monoacyladenines are extensively hydrolyzed, but maximum stability is still pH 5.1.

After complete hydrolysis the ultraviolet absorption spectrum was that of adenine (Curve 24, Figure 1). Paper chromatography also revealed the disappearance and replacement of monoacyladenine by adenine when monoacyladenines are hydrolyzed.

The position of the acyl group in monoacyladenines was established unambiguously with nitrous acid. Although no change in absorbance occurred at 248, 261, and 279 mμ with hypoxanthine and with the acyladenines, the reaction of adenine with HNO_2 resulted in a change in absorbance ratio E 248/261 mμ from 0.72 (zero time) to 1.40 (8 hr.). The absorbance ratio E 248/261 mμ of hypoxanthine at pH 6 in water is 1.38.[16] The characteristic ultraviolet absorption spectrum

(14) J. A. Montgomery, *J. Am. Chem. Soc.*, **78**, 1928 (1956).
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(16) G. H. Beaven, E. R. Holiday, and E. A. Johnson, in "The Nucleic Acids," Chargaff and Davidson, ed., Academic Press, New York, N. Y., 1955, Vol. I, p. 493.

TABLE I

ANALYSIS, CHROMATOGRAPHY, AND SPECTRAL CHARACTERISTICS OF 6-ACYLAMIDOPURINES[a]

Compound	Formula	M.p., °C.[a] Lit.	Ref.	Found	Carbon, %[b] Calcd.	Found	Hydrogen, %[b] Calcd.	Found	Nitrogen, %[c] Calcd.	Found	R_f Cmpd.	R_f Cmpd./ adenine	$E \times 10^{-3}$ at 279 mμ[d,e]	E 279/ 261 mμ
Acetyl[f]	$C_7H_7N_5O$	>280	11	350 dec.	47.45	47.80	3.95	4.02	39.1	39.0	0.58	2.0	13	1.61
		>260	12											
		>260	13											
Acetyl[g]				350 dec.		47.50		3.95		39.3			13	1.61
Propionyl[f]	$C_8H_9N_5O$	235–237	11	237–238	50.26	50.38	4.71	4.63	36.3	36.4	0.62	2.2	13	1.74
Butyryl[f]	$C_9H_{11}N_5O$	212–215	11	216–217	52.68	52.60	5.36	5.48	34.1	33.7	0.68	2.4	13	1.59
Butyryl[g]				216–217		52.60		5.40		34.5			13	1.59
Valeryl[f]	$C_{10}H_{13}N_5O$			211–213	54.79	54.70	5.94	5.90	31.9	31.2	0.73	2.6	13	1.73
Hexanoyl[f]	$C_{11}H_{15}N_5O$	202–204	10	200–202	56.65	56.85	6.43	6.43	30.0	29.6	0.73	2.6	13	1.62
Heptanoyl[f]	$C_{12}H_{17}N_5O$			187–189	58.29	58.22	6.88	6.75	28.3	28.3	0.76	2.7	13	1.75
Adenine[h]	$C_5H_5N_5$	360 dec.			44.4		3.73		51.8	51.6	0.28		14	0.11

[a] Melting points were taken in a constantly circulating, electrically heated silicone fluid bath and are uncorrected. Ascending chromatograms were run on cylinders of Whatman no. 1 paper with methanol as the developing solvent. In all cases only one spot was observed. The spots were located by visual examination with an ultraviolet lamp. Occasionally spots were eluted with methanol and the ultraviolet absorption spectra recorded on a Beckman DK-1 spectrophotometer. The spectra of the monoacyladenines were identical, nor was there any difference between the spectra of any of the monoacyl and diacyl derivatives of adenine. Adenine was run simultaneously with all samples for comparisons. [b] Carbon and hydrogen analyses were by Weiler and Strauss, Oxford. [c] Kjeldahl. [d] 2.6–4.4 × 10^{-5} M in methanol. [e] Max. = 279 mμ in methanol. [f] Prepared by aqueous hydrolysis of respective diacyladenine at 100°. [g] Prepared by treating respective diacyladenine with 1 equivalent of NaOH at room temperature. [h] Adenine employed as reference compound.

TABLE II

ANALYSIS AND CHROMATOGRAPHY OF 6-ACYLAMIDO-9(OR 7)-ACYLPURINES[a]

	Formula	M.p., °C. Lit.	M.p., °C. Ref.	Nitrogen, %[b] Found	Nitrogen, %[b] Calcd.	Nitrogen, %[b] Found	Chromatography, R_f Cmpd.	Chromatography, R_f Cmpd./adenine
Diacetyl	$C_9H_9N_5O_2$	195	11	193–196	31.9	31.4		
Dipropionyl	$C_{11}H_{13}N_5O_2$	180–182	11	145–148	27.9	27.8	0.65	2.3
Dibutyryl[c,d]	$C_{13}H_{17}N_5O_2$	152–154	11	158–160	25.5	25.6[e]	0.72	2.6
						25.4		
Divaleryl	$C_{15}H_{21}N_5O_2$			150–153	22.7	22.8	0.76	2.7
Dihexanoyl	$C_{17}H_{25}N_5O_2$			153–155	21.1	21.4	0.78	2.8
Diheptanoyl	$C_{19}H_{29}N_5O_2$			143–146	19.5	20.2	0.79	2.9
Adenine[f]	$C_5H_5N_5$	360 dec.			51.8	51.6	0.28	

[a] Melting point determinations and chromatography were carried out as previously described. Adenine was used as a reference standard. Spot locations are also expressed as the ratio R_f compd./adenine = 1. [b] Unless otherwise stated nitrogen was determined by the Kjeldahl method. [c] Calcd.: C, 56.7; H, 6.18. Found: C, 56.70; H, 6.35 (Weiler and Strauss). [d] Calcd.: O, 11.6. Found: O, 11.5 (Schwarzkopf Laboratories, New York City). [e] Dumas analysis (Weiler and Strauss). [f] Adenine employed as reference compound.

TABLE III

STABILITY STUDIES

Per cent. hydrolyzed[a]

pH	Hr. at 22°: 0.5	1	2	3	6	7	12	Hr. at 50°: 1	3	7	Hr. at 80°: 1	3	7	Hr. at 100°: 1	3	7
Butyryladenine																
5 *N* HCl							94									
1 *N* HCl			7		22		38									

TABLE III (*Continued*)

1.5					11	12	23	63	69	92	93	95	96	95
3.3					0	3	3	8	8	20	34	17	43	68
5.1					0	5	3	3	1	4	4	1	5	11
7.1					0	3	2	5	12	29	44	35	72	89
8.1					0	3	12	27	37	78	89	83	89	96
11.0					13									
12.4	4	13	31	62	88									
1 *N* NaOH	18	36	61	92	100									
5 *N* NaOH					100									
					Acetyladenine									
5 *N* HCl					100									
1 *N* HCl			8	26	50									
1.5					18	17	37	63	85	102	102	103	103	
3.3					5	1	3	4	9	24	43	25	59	83
5.1					5	0	4	0	4	4	7	4	11	22
7.1					0	0	3	3	10	20	35	53	96	102
8.1					5	5	12	27	65	97	100	99	105	
11.0					20									
12.4	13	24	48	76	100									
1 *N* NaOH	33	55	83	100	100									
5 *N* NaOH	[b]	[b]	[b]	[b]	100									

[a] The optical densities of acyladenine and adenine are 0.35 and 0.68, respectively, at 261 mμ. An increase in optical density of 0.01 at 261 mμ corresponds to 3% hydrolysis. Similarly, the optical densities are 0.68 and 0.08, respectively, at 279 mμ. A decrease in optical density of 0.01 at 279 mμ corresponds to 1.79 hydrolysis.

[b] Anomalous results; percentage hydrolysis calculated from changes in absorption at 261 and 279 mμ did not agree.

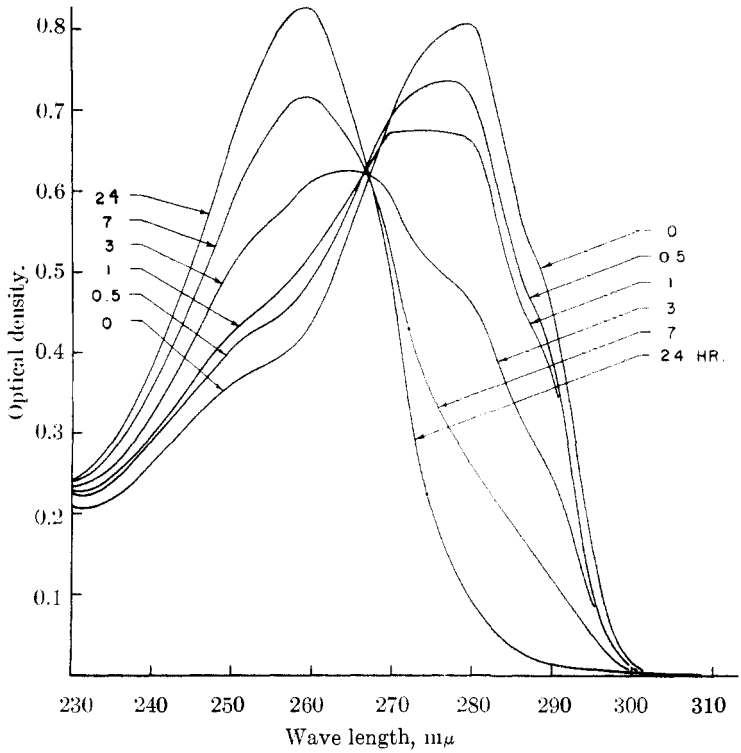

Fig. 1.—Hydrolysis of monobutyryladenine at pH 12.4, 22°, as a function of time—ultraviolet spectra. Conditions are described in the text.

of adenine disappeared in 8 hours and was replaced by the spectrum of hypoxanthine (Figure 2).

It is clear that the acyl group of monoacyladenines blocks the amino group on C-6 and renders it unsusceptible to nitrous acid deamination. The same results were obtained when adenine, hypoxanthine and acyladenines were treated with nitrous acid in a Van Slyke amino nitrogen apparatus. Adenine yielded the theoretical amounts of nitrogen gas while hypoxanthine and acyladenines did not react with nitrous acid.

Biological Section.—Homogenates and cell fractions of rat liver prepared in Versene-isotonic sucrose were incubated with monopropionyladenine in pH 6.8 phosphate buffer for 2 hr. at 37°. After freeing the solution of protein either by heating or with ethanol,

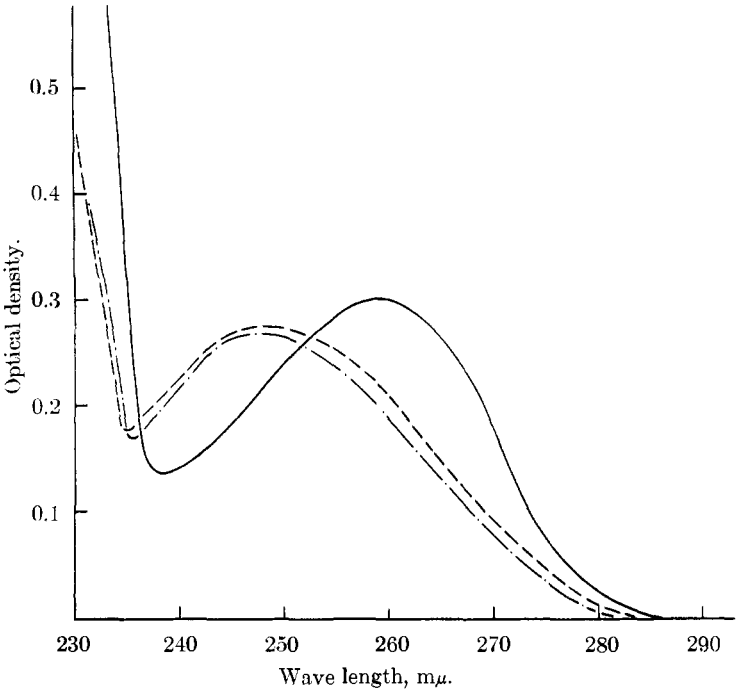

Fig. 2.—Deamination with nitrous acid: ——— adenine, 0 hr.; — — — adenine, 8 hr.; — · — hypoxanthine, 0 and 8 hr. Conditions are described in the text.

aliquots of the filtrates were removed for chromatography. Spots absorbing in the ultraviolet were cut out and eluted with methanol. Suitable enzyme (no substrate) and substrate (no enzyme) controls were simultaneously carried through the entire procedure. This was done under conditions which made possible quantitative comparisons between test and controls as measured by absorbance differences at λ 261 and λ 279. In addition the absorption between λ 220–300 of the protein-free filtrate was determined with a Beckman DU spectrophotometer.

Chromatography invariably yielded a single spot in the expected position for monopropionyladenine (R_f 2.2/adenine = 1). When this spot was eluted, the absorbance of the test at λ 261 and λ 279 was the same as the substrate control. Had hydrolysis occurred a fall in absorption at λ 279 should have been evident. The ultraviolet

spectra of the test and the substrate control protein-free filtrate were the same in shape and height indicating that no appreciable amount of monopropionyladenine had disappeared.

Altogether four experiments of this type were done under slightly varying conditions. In no case was there any evidence that rat liver cell fractions as well as homogenates of the intestinal mucosa, kidney, pancreas or spleen could hydrolyze monopropionyladenine. Similarly, homogenates of rabbit intestinal mucosa, kidney, spleen and pancreas, pigeon liver, pig heart and kidney, solutions or suspensions of trypsin, pepsin, chymotrypsin, papain, erepsin, pancreatin, bromelin, carboxypeptidase, acylase, ficin, lipase and bacterial extracts all failed to hydrolyze monopropionyladenine. Other monoacyl compounds were not tested.

The results with monopropionyladenine were considered sufficiently promising to warrant anticancer screening of all of the diacyl and monoacyladenines on mouse tumors, sarcoma 180, carcinoma 755 and leukemia 1210, at the Cancer Chemotherapy National Service Center. Dibutyryl and diheptanoyladenine were further screened for activity against the KB line of cell culture. Although some toxicity was noted, none of the compounds were sufficiently active to warrant further testing. Neither monopropionyl nor monoheptanoyladenine at oral doses of 75 mg./kg. exerted anti-implantation effects on pregnant rats.[17]

Experimental

6-Acylamido-9(or 7)-acylpurines.—N^6-9(or 7)-Diacyladenines were synthesized by the method of Birkofer.[11] Adenine (Nutritional Biochemicals Co.) was added to the hot fatty acid anhydrides in the molar proportion of 1:3-5 and the resulting clear solutions were stirred at 145–150° for 1 hr. under anhydrous conditions. On cooling, the reaction products solidified. Fatty acids and excess acid anhydrides were removed with petroleum ether (30–60°). The residues were extracted with boiling toluene. The precipitates from chilled toluene were filtered and dried in a vacuum oven over paraffin. Yields averaged 50–70%. The compounds were recrystallized from toluene.

Diacetyladenine may be more conveniently prepared by refluxing adenine with a large excess of acetic anhydride for 1 hr. The precipitate which appeared on cooling was filtered, washed with ethanol, recrystallized from toluene and dried over KOH in a vacuum oven at 45°. Yields averaged 60%. Additional diacetyladenine could be recovered from the acetic anhydride solution but two or three

(17) J. B. Bedford, personal communication, Worcester Foundation for Exptl. Biol., Shrewsbury, Mass.

recrystallizations from toluene were necessary before melting points and analytical data were satisfactory.

6-Acylamidopurines.—N^6-Acyladenines were prepared by two methods: (a) an aqueous suspension of a diacyl compound was boiled for 10–20 min. and filtered. The filtrate deposited crystals of monoacyladenine on cooling.[11,14,15] As the carbon chain length of the acyl radical increased, smaller yields of monoacyladenine were obtained by this method, and these only after prolonged heating.

(b) One equivalent of NaOH was added over the course of a few hours to a 5% aqueous suspension of a diacyladenine. The pH was maintained at 11 by addition of NaOH or HCl. The resulting clear solution was neutralized. Monoacyladenine precipitated on standing. Yields ranged from 40–60%. The ultraviolet absorption spectra of the various monoacyladenines are identical and are typified by curve 0, Figure 1.

Stability Study—Monobutyryl and Monoacetyladenines.—Freshly prepared 5×10^{-4} *M* monobutyryladenine was diluted with an equal volume of various buffers as well as with 1 *N* and 5 *N* HCl and NaOH at 22, 50, 80, and 100°. At specified intervals aliquots were removed, neutralized to pH 7 and diluted to 5×10^{-5} *M*. Monoacetyladenine is less soluble; the corresponding concentrations were half that of monobutyryladenine. Absorbance at 261 and 279 mμ was measured in a Beckman DU spectrophotometer. As hydrolysis proceeded there was a fall in absorbance at 279 mμ (maximum for acyladenines at pH 7) and a corresponding rise at 261 mμ (maximum for adenine at pH 7). Percentage hydrolysis was calculated from the absorbance changes at both wave lengths. Usually these checked within a few per cent. The results are tabulated in Table III. Ultraviolet spectral changes during the course of hydrolysis are shown in Figure 1. In this instance the final concentration of monobutyryladenine was 6×10^{-5} *M*.

Progress of hydrolysis also was followed by chromatography. Monobutyryladenine was incubated at 22° with pH 12.4 buffer. At zero, 0.5, 1, 3, 5, and 24 hr. aliquots of the solution were removed, neutralized and dried. The residue was dissolved in a small volume of water and spotted on Whatman no. 1 paper, using methanol as solvent for chromatography. Spots were visualized with an ultraviolet lamp. Adenine was used as a reference. At time zero only the very faintest absorption was noted in the region where adenine would be located. As hydrolysis progressed the adenine spot darkened and the butyryladenine spot decreased in density. Finally at 24 hr. only one spot, that of adenine, could be demonstrated.

Nitrous Acid Deamination.—Sodium nitrite (5 ml., 1.8 *M*) was added to 20 ml. of 2.84×10^{-3}*M* hypoxanthine, adenine, and each of the monoacyladenines in acetic acid (1.1 *N*, pH 2.4). The resulting pH of the purine-nitrous acid solution was 3.8. Monoacyladenines are not hydrolyzed at this pH. After 0, 3 and 8 hr. at 22°, 5 ml. aliquots of the nitrous acid solution were removed, neutralized and diluted to 250 ml. with water. The ultraviolet spectra were recorded with a Beck-DK-1 spectrophotometer (Figure 2).