may, in actuality, attack similar types of internal linkage.

There appears to be a considerable divergence in action between urea and guanidine hydrochloride, as regards to reversibility of the alkaline pH profile in the presence of high levels of these reagents. The action of guanidine in unfolding proteins is undoubtedly more profound, on a molar basis, than urea. A possible explanation is consequently that S-S bridges may be more exposed to attack by base in guanidine solutions and thereby suffer (irreversible) oxidative cleavage leading to irreversible conformational changes.[21,22]

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(22) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 583 (1959).

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, URBANA, ILL.]

The Chemistry of Triacanthine[1,2]

BY NELSON J. LEONARD AND JAMES A. DEYRUP[3,4]

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Evidence from chemical degradations and physical methods has indicated that the structure of *triacanthine* is 6-amino-3-(γ,γ-dimethylallyl)-purine (V). Use of the "exchange amination" reaction has been introduced in the structure proof, and the acid cleavage of substituted N-allylic adenines has been documented. A combination of ultraviolet spectral and dissociation constant data has now provided a general method for distinguishing between 3-, 7- and 9-substituted adenines. Triacanthine and several of its isomers have been synthesized by alkylation of adenine, the alkylation on N_3 representing an unusual departure from the hitherto expected course of purine alkylations. Ozonolysis studies of substituted N-allylic adenines have revealed a new feature potentially important when this degradation of hemiterpenic side chains is used as a means of structure proof. Finally, the formation of pyrotriacanthine chloride (XXV) provides a starting point for the study of allylic ring closures and rearrangements on the purine nucleus.

Prior to 1900 there was a period in which a variety of alkaloidal and pharmacological activity was ascribed to *Gleditsia triacanthos* L. The origin and demise of the interest in *Gleditsia* in this country are amusingly set forth in two editorials which appeared in the *American Journal of Pharmacy* of 1887.[5] Other early reports suggested the presence of alkaloidal material in species of *Gleditsia*,[6,7] and in 1954, Belikov, Bankowsky and Tsarev[8] reported the isolation of the alkaloid triacanthine from the young leaves of *Gleditsia triacanthos*, to which they assigned the formula $C_8H_{10}N_4$. This empirical formula appeared intriguing due to the high ratio of nitrogen to carbon, which is in contrast to most other alkaloidal formulas, and investigation of triacanthine was especially attractive in view of the abundance of *Gleditsia triacanthos* in the State of Illinois. We have repeated the described extraction procedure and have confirmed the finding of the Russian workers that the triacanthine content decreases rapidly as the leaf of this tree develops.

Analytical data on the free base did not permit a decision between formulas $C_8H_{10}N_4$ and $C_{10}H_{13}N_5$ for triacanthine, m.p. 228–229° (reported[8] 227–228°) but the analytical data for the triacanthine salts, hydrochloride, m.p. 232–234° dec. (reported[8] 218–219°), and picrate, m.p. 246° dec. (reported[8] 239–241°), gave clear preference for $C_{10}H_{13}N_5$. This formula was supported by titrimetric (211 ± 10; pK_a' 5.4 in 50% DMF) and mass spectral (203) values for the molecular weight. The composition was indicative of a total of seven double bonds or rings in triacanthine. The lack of any detectable optical activity suggested the absence of an asymmetric center in triacanthine. The ultraviolet spectrum of triacanthine showed λ_{max}^{EtOH} 273 mμ (ϵ 12,500), and acidification produced a shift to λ_{max}^{EtOH} 277 mμ (ϵ 18,300). The infrared spectrum (KBr) showed maxima at 3400 and 3240 cm.$^{-1}$ (N–H stretching) and 1682, 1630 and 1557 cm.$^{-1}$ (aromatic skeletal vibrations). The mass spectrum showed peaks at 203, 188 and 135. The peak at mass number 230 was assigned to the $C_{10}H_{13}N_5^+$ ion. The peak at mass number 188 was apparently due to the loss of a methyl group producing a $C_9H_{10}N_5^+$ ion. Consequently, triacanthine has to possess at least one methyl group, a fact confirmed in part by a Kuhn–Roth C-methyl determination. The fragmentation represented by the peak in the mass spectrum at 135 ($C_5H_5N_5^+$), which was suggestive of the presence in the original alkaloid of an aromatic ring system containing all five nitrogens, was realized in other reactions.

Triacanthine could be recovered unchanged after treatment with hot, aqueous potassium hydroxide (in which it was insoluble); in contrast to this base stability, triacanthine was easily cleaved to adenine (I) by strong acids. In the initial experiment, adenine hydrochloride was isolated in 56% yield after heating triacanthine in concentrated hydrochloric acid for eight hours at 80°. Subsequent experiments showed that shorter periods of heating were equally effective. Identification of this prod-

(1) This investigation was supported by a research grant (USPHS-RG5829) from the National Institutes of Health, U. S. Public Health Service.

(2) Taken from the Ph.D. Thesis of James A. Deyrup, University of Illinois, 1961; seminar presentation (J.A.D.) at the University of Zürich, Switzerland, November 4, 1961.

(3) Eli Lilly and Co. Fellow, 1958–1959.

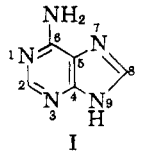
(4) National Science Foundation Co-operative Fellow, 1960–1961.

(5) *Am. J. Pharm.*, **59**, 541, 589 (1887).

(6) C. Wehmer, "Die Pflanzenstoffe," Verlag von Gustav Fischer, Jena, 1929, p. 508.

(7) M. Greshoff, "Mededeelingen uit 's Lands Plantentuin," **29**, 67 (1900), G. Kolff and Co., Batavia, 1900.

(8) A. S. Belikov, A. I. Bankowsky and M. V. Tsarev, *Zhur. Obsch. Khim.*, **24**, 919 (1954). A. S. Belikov and E. S. Zheleznova, *Trudy Vsesoyuz. Nauch.-Issledovatel. Inst. Lekarstv., i Aromat. Rast*, No. 1, 22–29 (1959) [*C. A.*, **55**, 20100 (1961)] have now given a partial structure, $C_8H_9N_4(CCH_3)NH$, for triacanthine, thus correcting their earlier empirical formula, $C_8H_{10}N_4$, in agreement with the present authors' $C_{10}H_{13}N_5$.



I

uct was made by means of melting point, analysis and ultraviolet spectrum. Conversion of the hydrochloride to the picrate produced material which failed to depress the melting point of authentic adenine picrate. It can be seen that this fragment contains all the nitrogen and half of the carbon atoms which were present in the original molecule. In order to establish the nature and position of attachment of the other fragment, triacanthine was subjected to the "exchange amination" reaction of Whitehead and Traverso.[9] Reaction of triacanthine with benzylamine and benzylamine hydrochloride in a sealed tube at 180° for eighteen hours yielded N-benzyltriacanthine, $C_{17}H_{19}N_5$, m.p. 150°. By a simple process of subtraction, it was seen that a $C_6H_5CH_2NH-$ group displaced an unsubstituted amino (or imino) group and it could be safely concluded that the adenine nucleus in the original was unsubstituted on the exocyclic nitrogen atom. In addition to this important fact, it was possible to obtain other useful information from N-benzyltriacanthine. Due to the benzyl group, N-benzyltriacanthine is significantly more soluble than triacanthine. For this reason, it was possible to obtain a suitable n.m.r. spectrum in deuteriochloroform. Although part of the spectrum was not clear due to the coincidence of the benzyl-CH_2- signal with other peaks in triacanthine, four peaks were clearly resolved. One of these was a sharp single peak at 2.77 τ. This value is in good agreement with that expected for the benzene ring aromatic hydrogens.[10] The highest peak in the spectrum occurred at a τ-value of 8.14. Since it has already been demonstrated that triacanthine contains at least one methyl group, this peak at 8.14 must be from the methyl hydrogens. More specifically, it could be concluded from the τ-value of 8.14 that this signal is due to a $CH_3—C=C$ group.[10] By means of a comparison of the area of the 8.14 peak with the area of the 2.77 peak as an internal standard (five phenyl hydrogens), after extrapolating to zero r.f. power,[11,12] we ascertained that triacanthine has two methyl groups attached to a double bond, since the hydrogen ratio was found to be 5.72:5. The lowest two peaks in the n.m.r. spectrum, at τ = 2.05 and 2.23, must derive from the two hydrogens attached directly to the purine nucleus. They are in good qualitative agreement with the values obtained by Jardetzky and Jardetzky[13] for adenosine at various *p*H's in D_2O. Accordingly, triacanthine is not substituted on C_2, C_8 or on the 6-amino nitrogen.

Further evidence for the presence of a double bond in the side chain attached to the adenine nucleus was found in the catalytic hydrogenation of triacanthine with hydrogen and platinum in glacial acetic acid to dihydrotriacanthine, having almost the same melting point and identical R_f values as triacanthine. The ultraviolet spectrum of dihydrotriacanthine showed λ_{max}^{EtOH} at 273 mμ (ϵ 13,020) and upon acidification at 277 mμ (ϵ 18,950). The similarity of the ultraviolet spectra of triacanthine and dihydrotriacanthine ruled against any structure which would permit overlap between a double bond and the purine ring. The low (42%) yield of dihydrotriacanthine was found to be due to the formation of adenine as a by-product of the hydrogenation. Triacanthine was unchanged when stirred with glacial acetic acid at room temperature for several days. Therefore, the adenine arose *via* hydrogenolysis and not acid cleavage. It is also significant that dihydrotriacanthine was unchanged when treated with strong acid in contrast to the ease with which triacanthine was converted to adenine under the same conditions. These results suggested that the double bond in triacanthine is so situated as to make the side chain a good leaving group, *i.e.*, allylic to one of the endocyclic nitrogen atoms. There is similarity in the acid cleavage and the hydrogenolysis of triacanthine with the behavior of the compound brayleyanin, also of natural source, in cleavage of the γ,γ-dimethylallylic side chain from oxygen.[14]

The three carbon atoms of the allylic system together with the two methyl groups account for the five carbons of the side chain, which in partial structure must be $(N)—CH_2C=C(—)_{2CH_3}^{1H}$. The location of the two methyl groups on the allylic system was easily and definitively resolved when it was discovered that liquid sulfur dioxide was an excellent solvent for determining the n.m.r. spectra of triacanthine and dihydrotriacanthine. These spectra are reproduced schematically in Figs. 1 and 2.[15] In the spectrum of triacanthine (Fig. 1), the

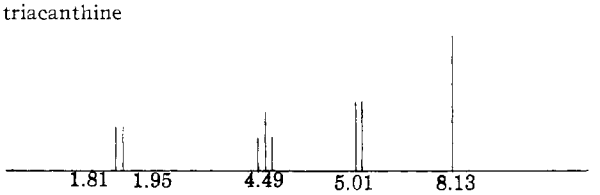


Fig. 1.

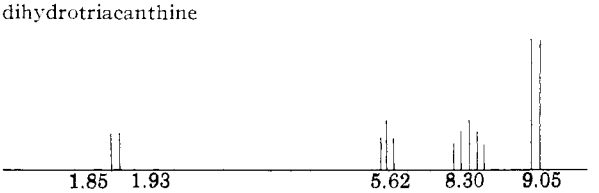


Fig. 2.

(9) C. W. Whitehead and J. J. Traverso, *J. Am. Chem. Soc.*, **82**, 3971 (1960).

(10) G. V. D. Tiers, "Table of τ Values for a Variety of Organic Compounds," Minnesota Mining and Manufacturing Co., St. Paul, Minn., 1958; G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(11) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 77.

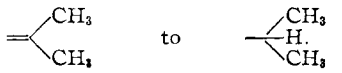
(12) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 49.

(13) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).

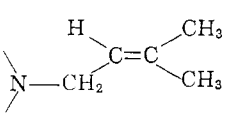
(14) F. A. L. Anet, G. K. Hughes and E. Ritchie, *Austral. J. Sci. Research*, **A2**, 608 (1949).

(15) See ref. 2 for complete spectra.

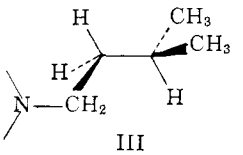
triplet at 4.49 and the doublet at 5.01 are characteristic of the $-CH-CH_2-$ group. The J-value for these two multiplets is 7.6. The equivalence of J for the two multiplets is additional evidence for their adjacency in the partial structure $-CH-CH_2-$. The splitting of the singlet methyl peak at 8.13 into a doublet at 9.05 in dihydrotriacanthine is only accommodated by the transition



The failure to observe a peak for the methynyl hydrogen is probably due to the difficulty in detecting a signal of the expected high multiplicity; it could also be obscured by the peak at 8.30. The signal at 5.62 in dihydrotriacanthine is assigned to the methylene group adjacent to the nitrogen. The n.m.r. results are thus accommodated by the side-chain structure II for triacanthine and III for dihydrotriacanthine. The possibility of structures containing a cyclopropane ring is also ruled out by these n.m.r. spectra. It is



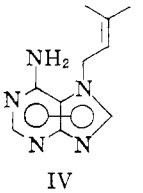
II



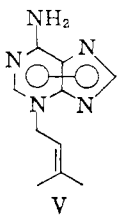
III

somewhat surprising that only one methyl peak is seen in the spectrum of triacanthine. Bates and Gale[16] have shown that in systems containing $X-CH_2CH{=}C(CH_3)_2$, in which X is one of a variety of substituents, the methyl groups are resolved into two peaks. Apparently, in the case of triacanthine and N-benzyltriacanthine, the summation of the inductive effects which are operative through the carbon skeleton and anisotropic effects which are not (and possibly others) coincidentally add up in such a way that the $X-CH_2-$ group provides the same electronic environment as the olefinic hydrogen.

The final problem concerning the structure of triacanthine was the position of attachment of the γ,γ-dimethylallyl side chain on the adenine nucleus. Since this problem proved more complex than envisaged at the outset and since some new principles were realized during the course of our experimentation, it is necessary to provide the background for the assignment of structures to N-substituted adenine derivatives. It has been tacitly assumed that the presence of a pK_a' in the region 8–11 is indicative of the >N–H group in the imidazole ring of a substituted adenine. Such a pK_a' suggests, therefore, that the imidazole ring is unsubstituted and that one of the other nitrogen atoms is substituted. An example of this is kinetin, which has a weak acid pK_a' of 10.[17] It has been similarly assumed that the absence of such a pK_a' in the 8-11 region indicates substitution at either N_7 or N_9. Examples of this may be found in the cases of adenosine,[18] puromycin,[19] the isomer of adenosine from pseudovitamin B_{12},[20] and finally triacanthine.[21] Application of this simple assumption in the latter case, however, together with a comparison of ultraviolet spectra of 7- and 9-substituted adenines, led to the preliminary assignment of an incorrect structure, 6-amino-7-(γ,γ-dimethylallyl)-purine (IV).[21] It can now be shown that more careful utilization of information from the dissociation constants in conjunction with



IV



V

ultraviolet spectral data allows assignment of structure V, 6-amino-3-(γ,γ-dimethylallyl)-purine, to triacanthine.

Since application of the information yielded by dissociation constants and ultraviolet spectroscopy is dependent upon comparison with model systems, it is necessary to examine the evidence for the structure of these models. Only in the case of the methyl derivatives have all five possible monosubstituted N-alkyl derivatives been prepared. Elion assembled the requisite data for 7-, 9- and 3-methyladenine and for 6-methylaminopurine, providing syntheses for the latter two derivatives.[22] New syntheses for 7-[23] and 9-methyladenine[24] have recently been developed, and the final isomer, 1-methyladenine, was obtained in 1960, among other products, by the reaction of adenosine with dimethyl sulfate in dimethylformamide.[25] By a process of elimination the structure of this final isomer was assigned, and as added support for the assignment it was demonstrated that upon heating the compound in concentrated aqueous ammonia, 6-methylaminopurine was obtained, as identified by ultraviolet spectrum and R_f-value. An unequivocal synthesis of 1-methyladenine has not yet been achieved. The ultraviolet spectra and melting points of the five methyladenines are presented in Table I. Inspection of this table reveals—and Elion has pointed out[22a]—that a great similarity exists in the spectral properties of the 3- and 7-isomers. As a consequence of this similarity, it follows that a monosubstituted adenine with an absorption maximum in the vicinity of 270 mμ in basic solution which shows both a bathochromic and hyperchromic shift upon acidification may be

(16) R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.*, **82**, 5749 (1960); R. B. Bates, private communication.

(17) C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza and F. M. Strong, *ibid.*, **78**, 1375 (1956).

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(19) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, *J. Am. Chem. Soc.*, **75**, 2025 (1953).

(20) W. Friedrich and K. Bernhauer, *Chem. Ber.*, **89**, 2507 (1956).

(21) N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, **82**, 6202 (1960).

(22) (a) G. B. Elion, "Ciba Foundation Symposium on the Chemistry and Biology of Purines," Ed. by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown and Co., Boston, Mass., 1957, p. 39; (b) G. B. Elion, E. Burgi and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952); (c) G. B. Elion, *J. Org. Chem.*, in press.

(23) R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6401 (1957).

(24) R. K. Robins and H. H. Lin, *ibid.*, **79**, 490 (1957).

(25) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960).

TABLE I

SUMMARY OF ULTRAVIOLET SPECTRA AND MELTING POINTS OF THE METHYLADENINES

Compound	M.p., °C. Base	M.p., °C. Sulfate	Ultraviolet spectra (aq. soln.) H^+ λ_{max}, mμ	H^+ ε	OH^- λ_{max}, mμ	OH^- ε
1-Methyladenine	.....	276–278[25]	259	11,700	270	14,400[25]
3-Methyladenine	300–302[a,c]	268–270[25]	274	17,000	273	13,300[22c]
6-Methylaminopurine	312–314[22b]		267	14,900	272	15,200[22b]
7-Methyladenine	351[23]		272	15,050	270	10,500[23]
9-Methyladenine	310[24]		260	14,200	260	14,700[b]

[a] Determined on a sample kindly donated by Dr. G. H. Hitchings, Burroughs Wellcome and Co., Inc., Tuckahoe, N. Y. [b] Robins and Lin[24] report λ_{max} 261 (14,600)(acid) and λ_{max} 262 mμ (11,900)(base). The latter printed ε is in error. Spectral data on a sample generously provided, along with 7-methyladenine, by Dr. R. K. Robins of Arizona State University, Tempe, Ariz., were in agreement with those given earlier by Gulland and Holliday.[26] [c] We find m.p. 309–311° for our synthetic 3-methyladenine, identical with that of Dr. R. K. Robins' sample obtained by the methylation of adenine.

either 3- or 7-substituted. On the basis of the ultraviolet spectra, triacanthine must be either IV or V. Prior to this work, no consideration had been given to the problem of distinguishing between 7- and 3-substituted adenines. An additional development was necessary in order to make this choice. A study of the infrared spectra yields little information; the inability to employ solutions for such a study makes this means of comparison especially unattractive. It was possible, however, to distinguish between 7- and 3-substitution by an additional comparison of the dissociation constants of triacanthine and the model compounds. It may be seen from Table II that triacanthine and dihydrotriacanthine possess dissociation constants which are in good agreement with the pK_a' that was determined for 3-methyladenine. The pK_a' for the 7-methyl isomer is quite different from that of 3-methyladenine and similar to that of 9-methyladenine. On the basis of these data, triacanthine should now be assigned the structure 6-amino-3-(γ,γ-dimethylallyl)-purine (V).

TABLE II

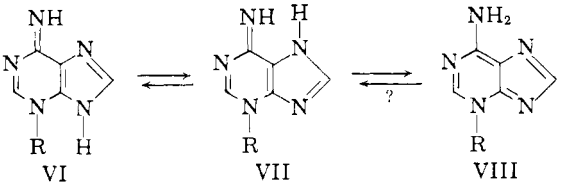
ACID DISSOCIATION CONSTANTS OF MONOSUBSTITUTED PURINES

Compound	pK'_a Titrimetric[a]		pK'_a Spectral[b]	
1-Methyladenine	6.95	11.9[c]	7.2	11.0[25]
3-Methyladenine	5.3			
6-Methylaminopurine	..			
7-Methyladenine	3.6			
9-Methyladenine	3.25			
Triacanthine	5.4		6.0	
Dihydrotriacanthine	5.2			
Adenosine	2.8			
7-D-Ribofuranosidoadenine	3.3[d]		3.9[20]	

[a] 50% DMF–50% water. The authors extend their thanks to Dr. Harold Boaz, Eli Lilly and Co., Indianapolis, Ind., for these important determinations. [b] Water. [c] Determined on a sample kindly furnished by Dr. P. D. Lawley, Pollards Woods Research Station, Chalfont St. Giles, Bucks, England. [d] Determined on a sample generously provided by Dr. W. Friedrich, Physiologisch-Chemisches Institut der Universitat Hamburg, Germany.

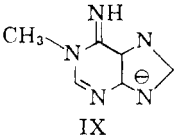
It is also of interest to note that the nucleoside from pseudovitamin B_{12} has a dissociation constant which is in agreement with the structure 7-D-ribofuranosidoadenine which was assigned to it.[20] The assignment of this 7-substituted adenine structure, which was made on the basis of ultraviolet spectroscopy, is thus corroborated by present comparison of its dissociation constant with those of 3- and 7-methyladenine.

The absence of a dissociation constant for triacanthine in the pK_a' range 9–12 suggests that there is no ionizable proton on the five-membered (imidazole) ring, consistent with its formulation as the tautomeric form V rather than either of the forms VI or VII (R = γ,γ-dimethylallyl). Moreover, it



VI VII VIII

appears that for a similar reason 3-methyladenine should be represented in its tautomeric form VIII (R = CH_3), rather than by the form VI (R = CH_3) which has appeared in the literature.[22a,25] In the case of 1-methyladenine, which has one pK_a' value at 11.0, attributed to the imidazole ring,[25] it may be concluded that the anion IX can be formed; however, the possibility of rearrangement of the initial compound by strong alkali has been indicated.



IX

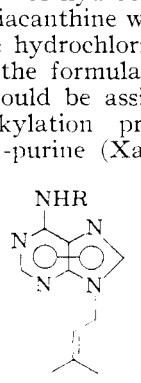
Synthesis of Triacanthine.—The sole method in the literature for the unambiguous synthesis of 3-substituted purines has been outlined by Elion,[22] and was applied to the synthesis of 3-methyladenine. Another method, by Denayer, Cavé and Goutarel,[27] promises to have broad scope and interest in purine chemistry, in addition to providing corroboration for the triacanthine structure (V) by synthesis. By application of the Elion method, we have synthesized 6-amino-3-isopentylpurine (VIII, R = $CH_2CH_2CH(CH_3)_2$) and found it identical, by the usual physical criteria including X-ray powder pattern, with dihydrotriacanthine.[28] Since we postulated originally that the γ,γ-dimethylallyl side chain in triacanthine was attached at N_7 to the

(26) J. M. Gulland and E. R. Holliday, *J. Chem. Soc.*, 765 (1936).

(27) R. Denayer, A. Cavé and R. Goutarel, *Compt. rend.*, **253**, 2994 (1961). The authors are grateful to Dr. Goutarel for providing a copy of this note prior to publication.

(28) Details will be provided in the sequel: N. J. Leonard and R. A. Laursen, *J. Org. Chem.*, in press.

purine nucleus,[21] our first synthesis of triacanthine followed a different route, patterned after methods which have been used for making 7- and 9-substituted purines.[29-32] The result was a synthesis of 6-amino-3-(γ,γ-dimethylallyl)-purine (V), triacanthine, which was novel but not unequivocal. A solution of pure γ,γ-dimethylallyl bromide in ethanol was added to the sodium salt of adenine (I) in ethanol at $-10°$ and the mixture was eventually heated at reflux, yielding a product of melting range 188–196°. Since N-benzyltriacanthine possesses greater solubility than triacanthine itself (see above), we took advantage of this property in the first separation of products. The crude synthetic mixture was caused to react with benzylamine and its hydrochloride to convert the whole to the N-benzyl derivative. Separation of the N-benzylated product was accomplished by chromatography on alumina. Elution with benzene yielded N-benzyltriacanthine, $C_{17}H_{19}N_5$, identical with authentic material in every respect (m.p., mixed m.p., X-ray powder pattern, ultraviolet spectrum, etc.). Continued elution of the column furnished an isomer of N-benzyltriacanthine, m.p. 163.3–163.8°; λ_{max}^{EtOH} (neutral) 278 mμ (ϵ 15,780), (pH 1) 285 mμ (ϵ 20,600). Hydrogenolysis of the γ,γ-dimethylallyl and benzyl groups appeared to proceed at about the same rate, so that in order to effect a synthesis of triacanthine it was necessary to separate the original alkylation mixture, without recourse to the N-benzylation step. The alkylation mixture was initially divided, by a single fractional crystallization, into two portions. These two fractions were then chromatographed separately on a column containing 750 g. of deactivated alumina for each gram of material applied. Elution with 10% ethanol in chloroform yielded the first component. After further elution with 25% ethanol in chloroform, a second component was obtained. In this manner, a clean separation was possible. Due to the large excess of alumina, only 60% of the original material was recovered from the column. The second component was identical with triacanthine in melting point, mixed-melting point, infrared spectrum, and ultraviolet spectra at different pH's. A hydrochloride prepared from this synthetic triacanthine was identical with authentic triacanthine hydrochloride. The first component, which had the formula $C_{10}H_{13}N_5$, m.p. 167–168°, pK_a' 3.3, could be assigned the structure of the *expected* alkylation product, 6-amino-9-(γ,γ-dimethylallyl)-purine (Xa), on the basis of the pK_a'



Xa, R = H
b, R = $CH_2C_6H_5$

and the ultraviolet spectra, neutral and acid. The isomer of N-benzyltriacanthine of m.p. 163.3–163.8° obtained from the same alkylation mixture is therefore 6-benzylamino-9-(γ,γ-dimethylallyl)-purine (Xb). Of the material actually isolated from the chromatography column, 65% was triacanthine. On the basis of the ultraviolet spectra of the triacanthine, Xa and the original alkylation mixture, it can be calculated[33] that close to 70% of the mixture was triacanthine. The *unexpected* feature of the alkylation was that it had taken place on N_3 and N_9 instead of N_7 and N_9.[29-32]

When the alkylation was carried out by adding a solution of the sodium salt of adenine in ethanol to an ether solution of γ,γ-dimethylallyl chloride (prepared from γ,γ-dimethylallyl alcohol[34] and phosphorus trichloride and used without further purification) at $-70°$, and eventually heating at reflux, a low yield of chloroform-soluble, alkali-insoluble $C_{10}H_{13}N_5$ compound, m.p. 195–196°, was obtained which could be purified by sublimation. The n.m.r. spectrum of this isomer in liquid sulfur dioxide was like that of triacanthine; the pK_a' of 3.2 and the shift toward higher extinction and longer wave length (see Table III) permitted the assignment of the structure 6-amino-7-(γ,γ-dimethylallyl)-purine (IV). The properties of the three synthetic γ,γ-dimethylallylation products of adenine are assembled in Table III, and it will be seen by comparison of the pK_a' values and the ultraviolet spectral maxima with those of the methyladenine isomers given in Tables I and II that the structure assignments are all consistent.

Thus, preliminary results indicate that with "reactive" halides, alkylation can be directed to either N_7 or N_3 and N_9. The necessary "reactivity" of the alkylating agent is yet to be defined. The influence of solvent, steric factors, pH, concentration and temperature should be reinvestigated in order to demonstrate the full scope of these relatively old synthetic procedures. Such an investigation currently under way in this Laboratory includes direct alkylation of adenine with sugar derivatives.[2]

It was necessary, however, to prepare some substituted purines in connection with other problems in the chemistry of triacanthine. For example, it was possible to alkylate the sodium salt of adenine with two other allyl chlorides under the same conditions (ethanol–ether at $-70°$ and eventual refluxing) as employed in the synthesis of 6-amino-7-(γ,γ-dimethylallyl)-purine (IV). Reaction of XI and XIII with the sodium salt of adenine led to two different isomeric $C_{10}H_{13}N_5$ substances, XII and XIV. The structure of the side chain in these two compounds was established by n.m.r. spectroscopy. The retention of configuration about the double bond follows from the work of Bates, who has shown that allylic halides prepared from geraniol and nerol do not isomerize appreciably when used to alkylate the sodium salt of acetoacetic ester under these conditions.[16] The location of the side chain was demonstrated by ultraviolet spectroscopy and dissociation constant data (see Table III).

(29) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *ibid.*, **19**, 1780 (1954).

(30) B. R. Baker, R. E. Schaub and J. P. Joseph, *ibid.*, **19**, 638 (1954).

(31) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).

(32) H. J. Schaeffer and R. D. Weimar, Jr., *ibid.*, **81**, 197 (1959).

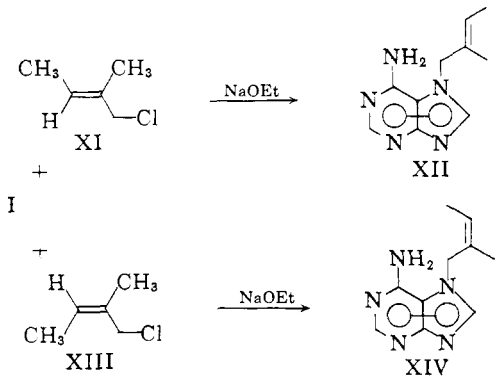
(33) M. J. S. Dewar and D. S. Urch, *J. Chem. Soc.*, 345 (1957).

TABLE III

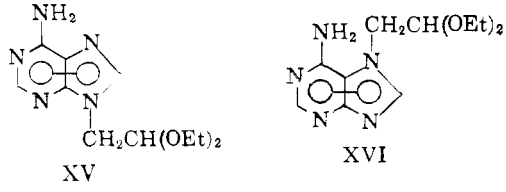
SUMMARY OF ADENINE ALKYLATION PRODUCTS

Compound, 6-amino-purine	M.p., °C.	M.p. HCl, °C.	pKa' (50% DMF)	U.v. spectra H+ λ_{max}, mμ	H+ ε	Neutral λ_{max}, mμ	Neutral ε
3-(γ,γ-Dimethylallyl)- (V) (triacanthine)	228–229	232–234	5.4	277	18,300	273	12,500
7-(γ,γ-Dimethylallyl)- (IV)	195–196	203–205	3.2	276	14,600	272	9,750
9-(γ,γ-Dimethylallyl)- (Xa)	167–168	247–248	3.3	260	14,000	260	13,800
7-(*cis*-β,γ-Dimethylallyl)- (XII)	250	216–218	3.2	276	14,400	272	9,200
7-(*trans*-β,γ-Dimethylallyl)- (XIV)	214	.....	..	276	14,400	272	9,200
9-(β,β-Diethoxyethyl)- (XV)	217–218	.....	..	259	14,570	260	14,700
7-(β,β-Diethoxyethyl)- (XVI)	166–168	.....	3.3	275	15,350	271	9,350

It was also desirable, for reasons to be stated below, to cause the sodium salt of adenine to react with bromoacetaldehyde diethyl acetal.[34]



Additonal interest was attached to this reaction since it involved alkylation with a very unreactive halide. As a consequence of this lack of reactivity, longer reaction time and higher temperature were necessary. The products of this reaction were also separated by chromatography. The major product, m.p. 217–218°, which had the empirical formula $C_{11}H_{17}N_5O_2$, was assigned structure XV on the basis of its ultraviolet spectra. A second, isomeric product was also isolated, m.p. 166–168°, which, from a consideration of its ultraviolet spectra, was either the N_3- or N_7-alkylated isomer. Inspection of pK_a' values indicated that it was the N_7-isomer XVI. Apparently, on the basis of limited evidence, for alkylation to occur at N_3 relatively mild conditions are required.



Further Consideration of Ultraviolet Spectra.—In view of the differences and similarities in the structures of the N_3-, N_7- and N_9-substituted adenines, it would be surprising if the ultraviolet spectra did not show corresponding differences and similarities. A closer inspection of the ultraviolet absorption spectra collected in this study[2] has revealed that a subtle but useful difference exists between the spectra of the N_7- and N_9-substituted adenines on one hand and the N_3-substituted adenines on the other. The value of λ_{min} (*p*H 1) − λ_{min} (*p*H 7) is *positive* for N_7- and N_9-substituted adenines and *negative* for the N_3-substituted. The differences which are given in Table IV are subject to the usual errors, but the decision as to whether the difference lies on one side or the other of zero is a clear one.

TABLE IV

$\Delta\lambda_{min}$ FOR N_3-, N_7- AND N_9-SUBSTITUTED ADENINES

Compound	λ_{min} (*p*H 1) − λ_{min} (*p*H 7), mμ
6-Amino-3-(γ,γ-dimethylallyl)-purine (triacanthine)	−8
6-Amino-3-isopentylpurine (dihydrotriacanthine)	−8
3-Methyladenine	−7[a]
6-Amino-7-(γ,γ-dimethylallyl)-purine	+11
7-Methyladenine	+7[b]
6-Amino-7-(*cis*-β,γ-dimethylallyl)-purine	+9
6-Amino-7-(*trans*-β,γ-dimethylallyl)-purine	+8
6-Amino-7-(β,β-diethoxyethyl)-purine	+3
6-Amino-9-(γ,γ-dimethylallyl)-purine	+4
9-Methyladenine	+3[c]
6-Amino-9-(β,β-diethoxyethyl)-purine	+5
6-Benzylamino-3-(γ,γ-dimethylallyl)-purine (N-benzyltriacanthine)	−6
6-Benzylamino-9-(γ,γ-dimethylallyl)-purine	+6
7-D-Ribosyladenine	+6[d]

[a] 237 − 244 mμ. [b] 239 − 232 mμ. [c] 230 − 227 mμ. [d] From spectral curves in ref. 20.

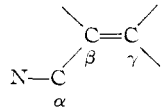
Inspection of the curves is also helpful.[15] The criterion is valid for the compounds which have been studied in this Laboratory (including the N-benzyl derivatives), and generality is predictable within these compound types. This additional guide for structure assignment in the purine series may be used in conjunction with the comparison of isosbestic points.[20,27]

Ozonolysis Studies.—The ozonolysis of triacanthine, investigated at an early stage in the structure determination, originally presented considerable ambiguity which was only resolved by the n.m.r. indication of the γ,γ-dimethylallyl side chain (II) and by ozonolysis experiments with model compounds. The results are presented not so much for their corroboration of the structure of the triacanthine side chain as for their novelty and the potential future value of the aberrancies that were encountered. N-Benzyltriacanthine was first ozonized in acetic acid–acetic anhydride at −70°. After reduction of the ozonide solution with zinc dust, the low molecular weight carbonyl components were isolated by steam distillation into a solution of 2,4-dinitrophenylhydrazine. The DNP derivative which was isolated in this manner was *acetaldehyde*

(34) H. Baganz and E. Brinckmann, *Chem. Ber.*, **86**, 1318 (1953).

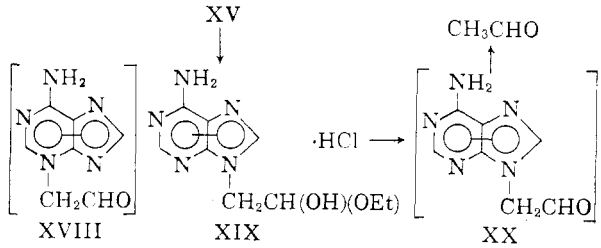
2,4-dinitrophenylhydrazone (55% yield). Ozonization of triacanthine at 25° in the same solvent system yielded the same product in somewhat lower yield. Neither sample of the DNP derivative of acetaldehyde contained detectable (by infrared) amounts of acetone 2,4-dinitrophenylhydrazone.

In order to check these potentially misleading results, 6-amino-7-(*cis*-β,γ-dimethylallyl)-purine (XII) was subjected to ozonolysis and reductive work-up. *Acetone*, as the DNP derivative (contaminated with approximately 15% of acetaldehyde 2,4-dinitrophenylhydrazone) was identified as the major product! It was therefore clear that in the system XVII, the α- and β-carbons, with their substituents, were constituting the corresponding carbonyl derivative, while the γ-carbon, and its substituents, were being converted mainly into something other



XVII

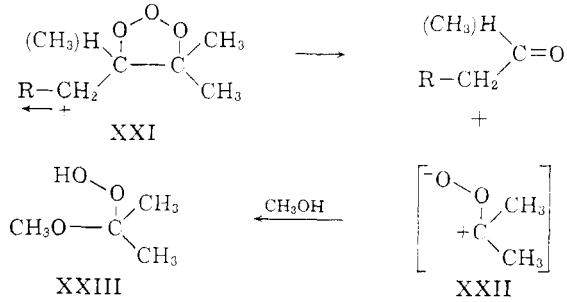
than a ketone or an aldehyde. Formation of acetaldehyde from the α- and β-carbons in triacanthine (V) could be accounted for by reductive deamination of the intermediate aldehyde XVIII. A vinylo-



XVIII XIX XX

gous example of this type of cleavage is found in the deamination of oxytetracycline by zinc dust in acetic acid to desdimethylaminoterramycin.[35] In order to demonstrate that cleavage of the system in XVIII actually does take place under the conditions of the ozonolysis work-up, the acetal XV was prepared. The synthesis of this compound was presented earlier in the discussion. Mild hydrolysis (1 *N* hydrochloric acid at 80° for seven minutes) of this acetal yielded the hemiacetal hydrochloride XIX. The structure of the hemiacetal was assigned tentatively on the basis of an ultraviolet spectrum, which was characteristic of a 9-substituted adenine, and analytical data. The stability of this hemiacetal is similar to that of hemiacetals of chloral and appears to be due, as in the case of chloral hemiacetals, to an inductive effect. The protonated adenine ring exerts a strong electron-withdrawing effect on groups attached to one of the endocyclic nitrogen atoms.

Attempts to isolate the aldehyde XX by more vigorous acid hydrolyses were unsuccessful in spite of wide variations in the conditions which were employed. It was apparent that the aldehyde is too reactive to survive the conditions of the hydrolysis. For this reason, zinc dust was added to a solution of the acetal XV in aqueous acetic acid. The mixture was heated for one hour. Steam distillation of the solution allowed the isolation of acetaldehyde 2,4-dinitrophenylhydrazone. In this manner the zinc dust cleavage of the system in XVIII, protonated, was established. The initial cleavage of the ozonide from triacanthine (protonated) to XVIII and the acetone peroxide moiety would be consistent with the finding of Criegee[36] and of Bailey[37,38] that in a system like XXI, the aldehyde (ketone) group appears predominantly on the side of the electron-withdrawing substituent. The fate of the zwitterion peroxide XXII is a function of the solvent. The nature of the action of acetic anhydride on the supposed intermediate XXII, which was one of interference insofar as the final isolation of acetone



XXI XXIII XXII

was concerned, has not yet been determined.[2] When the ozonolysis of triacanthine was carried out in methanol, *acetone* 2,4-dinitrophenylhydrazone was isolated in good yield, followed by the more soluble *acetaldehyde* DNP derivative. Ozonolysis of triacanthine in acetic acid–methanol solution gave practically the same result. One role of methanol as a solvent in ozonolysis is to react with the zwitterion to form the methoxyhydroperoxide XXIII, which is then reducible to the ketone.[38,39] Our own experience with the solvent-dependence in the ozonide decomposition and with the hydrogenolysis of the N–Cα bond in the work-up suggests further experimentation and provides, moreover, a note of caution to others who may wish to apply ozonolysis in structural analysis of similar compounds.

Pyrotriacanthine.—One of the more interesting facets in the chemistry of triacanthine was the formation of pyrotriacanthine. Triacanthine itself appears to be stable to heat. It can be melted without decomposition and sublimed at atmospheric pressure. Triacanthine hydrochloride, however, melts with decomposition and evolution of gas. The gas had a distinctive odor which resembled that of isoprene. This was confirmed by repeating the pyrolysis on a larger scale and isolating isoprene as its Diels–Alder adduct with tetracyanoethylene (XXIV). This adduct was identical with the product formed from an authentic sample of isoprene and tetracyanoethylene. The mechanism of formation probably involves N_3–Cα cleavage followed by

(35) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

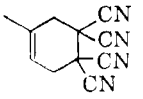
(36) R. Criegee, S. S. Bath and B. von Bornhaupt, *Chem. Ber.*, **93**, 2891 (1960).

(37) P. S. Bailey, S. B. Mainthia and C. J. Abshire, *J. Am. Chem. Soc.*, **82**, 6136 (1960).

(38) P. S. Bailey, *Chem. Revs.*, **58**, 927 (1958).

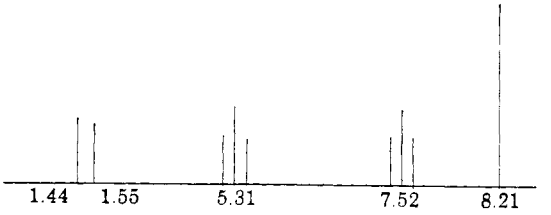
(39) R. Criegee, G. Blust, G. Lohaus, P. deBruyn and M. Lederer, *Ann.*, **583**, 1 (1953).

transfer of a proton from the isoprene-conjugate acid.

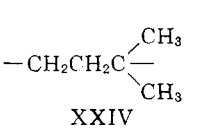


XXIV

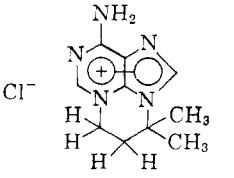
If isoprene is formed in the pyrolysis, adenine hydrochloride must be a by-product. Paper chromatography of the residue showed the presence in the pyrolysis residue of adenine hydrochloride plus another product which had a low R_f value (in contrast to triacanthine, dihydrotriacanthine, etc.) and gave a very intense Dragendorff test. The solubility of this substance in water implied that it was a salt. In view of the manner of its formation, it was named "pyrotriacanthine chloride." Advantage was taken of the high solubility of this salt in ethanol to separate it from the ethanol-insoluble adenine hydrochloride. Analytical data were in agreement with the formula $C_{10}H_{14}ClN_5$ and indicated that pyrotriacanthine chloride was isomeric with triacanthine hydrochloride. The most useful information regarding the structure of this product was obtained from an n.m.r. spectrum in liquid sulfur dioxide. This spectrum is reproduced schematically in Fig. 3. In this spectrum, it can be seen that the J-values (6.5) of the two triplets at 5.31 and 7.52 are identical. These two triplets may be assigned on this basis to two adjacent methylene groups. This fact, in addition to the singlet methyl



peak at 8.21, suggests the partial structure XXIV for pyrotriacanthine chloride. The low τ-value for the C–methyl hydrogens probably precludes ring closure to a carbon (rather than to N). Thus, in the absence of a deep-seated rearrangement, it is possible to incorporate XXIV into the cyclic structure XXV for pyrotriacanthine chloride. Formation of this product could proceed by the protonation of the triacanthine double bond to form the 3° carbonium ion, followed by rapid cyclization to XXV between carbonium carbon and nucleophilic N_9. Alternative mechanisms may also be envisaged. In this regard, it is instructive to compare the structure XXV with a related product derived from adenosine. Clark, Todd and Zussman[40] found that

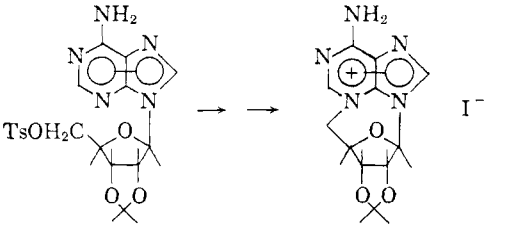


XXIV



XXV

the tosylate of 2′,3′-isopropylidene-adenosine (XXVI), on heating in acetone, was converted to an isomeric product. A further conversion to the iodide provided a salt suitable for X-ray analysis which established the structure as that of a *cyclo*-nucleoside (XXVII).[40,41]



XXVI XXVII

The ultraviolet spectrum of the cyclonucleoside XXVII showed λ_{max} at 272 mμ (ϵ 14,900), which compares favorably with the spectrum of pyrotriacanthine chloride (λ_{max} 275 mμ (ϵ 11,500)). The ultraviolet spectra of pyrotriacanthine chloride at other *p*H values indicated that pyrotriacanthine undergoes further reaction in basic solution. Thus, upon basification, maxima appeared at 258, 276 and 339 mμ. Reacidification did not produce the original spectrum. One conceivable reaction of pyrotriacanthine in basic solution would be ring opening to give a 3- and/or a 9-substituted adenine derivative. Attempts to characterize such a product, which should be readily isolated, were not successful. It is possible, therefore, that products were formed from attack by hydroxide ion on the purine ring, with subsequent degradation of the purine ring.[42]

Occurrence of Triacanthine.—Subsequent to our publication on the structure of triacanthine in 1960,[21] we learned that two other research groups had isolated natural bases of formula $C_{10}H_{13}N_5$. Janot, Cavé and Goutarel,[43] in 1959, reported the isolation of "togholamine" along with two steroidal alkaloids. The source was *Holarrhena floribunda* (G. Don) Dur. and Schinz, collected in Togo. Togholamine was shown to have the formula $C_{10}H_{13}N_5$. Infrared and ultraviolet spectral data were presented along with the pK_a' of the free base. Based on this information, togholamine was postulated as an aminopurine. In 1960, Monseur and Adriaens[44] published on the isolation of "chidlovine" and a terpene from *Chidlowia sanguinea* Hoyle, obtained from the Ivory Coast. The alkaloid was found to be distributed throughout the plant. The highest concentration was in the leaves (2%). Chidlovine was shown to have the formula $C_{10}H_{13}N_5$. On the basis of their ultraviolet and infrared spectral data, the Belgian workers postulated that chidlovine possessed a pyrimidine-type structure.

By means of a comparison of melting points, mixed-melting points and other physical data, it has now been shown that togholamine, chidlovine

(40) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(41) J. Zussman, *Acta Cryst.*, **6**, 504 (1953).

(42) B. R. Baker and J. P. Joseph, *J. Am. Chem. Soc.*, **77**, 15 (1955).

(43) M.-M. Janot, A. Cavé and R. Goutarel, *Bull. soc. chim. France*, 896 (1959).

(44) X. G. Monseur and E. L. Adriaens, *J. pharm. Belg.*, 279 (1960).

and triacanthine are identical.[45] Since the name *triacanthine* was applied to the first material to be isolated,[8] it is suggested that this name be used instead of chidlovine or togholamine.[45]

Although *Chidlowia* and *Gleditsia* both belong to the *Caesalpiniaceae* family, *Holarrhena* belongs to the *Apocynaceae* family. It is extremely interesting that triacanthine appears in two such distantly related families.[46] In *Gleditsia triacanthos*, triacanthine is present in insignificant concentrations during the greater part of the growing season. For this reason triacanthine had been overlooked in previous investigations. A similar situation may exist in many other plants. It is possible, therefore, that careful investigation would reveal small amounts of triacanthine (or substances of related origin) in a wide variety of plant material. The structure of triacanthine has been shown to be a novel combination of the ubiquitous biochemical structural units—adenine and isoprene. The γ,γ-dimethylallyl group has been previously found on nitrogen in galegine,[47] spherophysine[48] and smirnovine,[48] and attached to oxygen and carbon in many naturally occurring materials.[21] It appears likely that adenosine serves as the precursor of the adenine portion of triacanthine,[49] and γ,γ-dimethylallyl pyrophosphate,[50,51] the precursor of the hydrocarbon moiety. It is postulated that triacanthine is a metabolic by-product of the period of rapid cell division. It is open to question whether the formation of triacanthine by the plant is a mechanism of growth regulation.

Activity.—Although testing has not been completed, there has been no indication that triacanthine will exhibit high levels of pharmacological activity, on the basis of the information provided by the Smith Kline and French Laboratories and Eli Lilly and Co. Goutarel, Quevauvillier and Blanpin[52] have reported the results of their pharmacological testing independently. Professor F. Skoog of the University of Wisconsin reported to us that triacanthine hydrochloride tested in concentrations of 1 and 10 mg./l. in nutrient media without kinetin[17] showed no growth-promoting activity in either tobacco or soybean tissues which give clear-cut responses to kinetin, nor did these concentrations influence the growth obtained in the presence of either kinetin or adenine. In feeding tests by Professor G. S. Fraenkel, University of Illinois, on leaf-feeding insects (Colorado potato beetle, tobacco hornworm), triacanthine hydrochloride had no insect repellency.

Acknowledgments.—We enjoy recording our appreciation individually to the many people who aided us in this investigation: Professors W. R. Boggess and A. R. Gilmore of the Department of Forestry, University of Illinois, for helping us with plant procurement, and to Drs. Irwin Pachter and Bryce Douglas of the Smith Kline and French Laboratories, Philadelphia, Pa., for providing additional plant material; to Professor G. N. Jones, Department of Botany, University of Illinois, for botanical identification and taxonomy; to Professor Klaus Biemann of the Massachusetts Institute of Technology for determination of the mass spectrum of triacanthine; to the Smith Kline and French Laboratories and to Eli Lilly and Company for pharmacological testing; to Professor F. Skoog, University of Wisconsin, for bioassay for kinetin activity; to Professor G. S. Franekel, Department of Entomology, University of Illinois, for insect repellency and toxicity data; to Dr. Harold Boaz of Eli Lilly and Company, Indianapolis, Ind., for his special attention to the electrometric titrations which provided us with the crucial pK_a' data; and again to the chemists who have been cited in the text for providing us generously with samples and information.

Experimental[53]

Isolation of Alkaloidal Material from *Gleditsia triacanthos*.—Leaf material, in which the entire stem with young leaves had developed approximately two inches from the leaf bud, was collected by one of us (J.A.D.) in early spring from *Gleditsia triacanthos* growing at Dixon Springs, Ill. A 350-g. sample of this material, which had been ground and dried, was stirred for 24 hours with a mixture of 15 l. of chloroform, 250 ml. of ethanol and 150 ml. of 15% aqueous ammonia. After filtration, the residue was extracted with the same solvent mixture for 24 hours. The combined filtrates were concentrated *in vacuo* to approximately 1 liter. This concentrate was then extracted several times with 2 *N* sulfuric acid. The combined aqueous layers were then neutralized with concentrated aqueous ammonia (with cooling) and extracted four times with chloroform. The combined chloroform extracts were dried over magnesium sulfate, filtered, and evaporated to dryness to give 2.4 g. (0.69%) of total alkaloidal material.

More mature leaf material was collected approximately 2 months later in the growing season. In the same manner as above, 200 g. of ground, dried leaf material was extracted to give 40 mg. (0.02%) total alkaloidal material. At the same time, only very small, immature leaves (of the same approximate size used in the first extraction) were selectively collected. Extraction of 20.76 g. of this dried leaf material yielded 130 mg. (0.63%) of total alkaloidal material.

(45) A. Cavé, J. A. Deyrup, R. Goutarel, N. J. Leonard and X. G. Monseur, *Ann. pharm. fr.*, in press.

(46) Alternatively, according to an older method of classification, *Chidlowia* and *Gleditsia* both belong to the family *Leguminosae* (subfamily *Caesalpinoideae*). Regardless of classification system, morphological characteristics of *Chidlowia* and *Gleditsia* are distinctly different from those of *Holarrhena*. The authors are grateful to Professor G. N. Jones of the University of Illinois Botany Department for his helpful discussions concerning the classification of these plants.

(47) G. Barger and F. D. White, *Biochem. J.*, **17**, 827 (1923).

(48) A. J. Birch, D. G. Pettit and R. Schofield, *J. Chem. Soc.*, 410 (1957); see also A. J. Birch, P. Elliott and A. R. Penfold, *Austral. J. Chem.*, **7**, 169 (1954).

(49) The percentage of nucleic acids is greatest in the undeveloped leaves of plants; *e.g.*, see M. Holden, *Biochem. J.*, **51**, 433 (1952), esp. p. 441.

(50) S. Chaykin, J. Law, A. H. Phillips, T. T. Tchen and K. Bloch, *Proc. Natl. Acad. Sci. U. S.*, **44**, 998 (1958).

(51) B. W. Agranoff, H. Eggerer, U. Henning and F. Lynen, *J. Am. Chem. Soc.*, **81**, 1254 (1959).

(52) R. Goutarel, A. Quevauvillier and O. Blanpin, *Compt. rend. soc. biol.*, **155**, 470 (1961), in press. The authors appreciate receiving a copy of the manuscript before publication.

(53) Melting points are corrected; boiling points are uncorrected. All paper chromatographies were developed with the organic layer formed from a mixture of 75 ml. of 1-butanol, 20 ml. of water and 5 ml. of glacial acetic acid. Development was performed by the ascending method in a chamber saturated with solvent vapor. Comparison of samples were made on the same sheet and not by means of R_f value. Spots were detected by means of the Dragendorff spray and/or ultraviolet absorbance.

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Triacanthine (V).—Paper chromatography showed that all of the above alkaloidal extracts were identical (R_f value 0.75). Crystallization of the extracts from ethanol yielded colorless prisms, m.p. 228–229°. Paper chromatography of the mother liquors showed the presence of a very minor constituent. An analytical sample was prepared by sublimation of triacanthine at 160° (0.1 mm.), m.p. 228–229° (reported[8] 227–228°), $\alpha^{25}D$ 0° (ethanol), pK_a' (50% DMF) 5.4.

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45; N, 34.46; C-CH_3 (2), 14.80; mol. wt., 203.24. Found: C, 59.26, 59.30; H, 6.32, 6.31; N, 34.59; C-CH_3, 5.73; mol. wt., 211 ± 10 (titrimetric), 203 (mass spectral).

The ultraviolet spectra of triacanthine showed λ_{max}^{EtOH} (neutral) 273 (12,500), and (pH 1) 277 mμ (ε 18,300); λ_{max}^{EtOH} (neutral) 247 and (pH 1) 239 mμ. The infrared spectrum exhibited maxima at 3400 and 3240 (N–H stretching) and 1682, 1630, 1557 cm.$^{-1}$ (aromatic skeletal vibrations). The mass spectrum of triacanthine showed peaks at mass 203 (due to the $C_{10}H_{13}N_5^+$ ion), mass 188 (due to a $C_9H_{10}N_5^+$ ion—indicative of the loss of a CH_3– group) and mass 135 (probably due to a $C_5H_5N_5^+$ ion).

Triacanthine hydrochloride was prepared from a solution of 58.7 mg. of triacanthine in 2 ml. of absolute ethanol which was cooled to 0° and saturated with anhydrous hydrochloric acid. Filtration of the crystalline solid yielded 59.7 mg., m.p. 231–233° dec. Recrystallization from ethanol yielded an analytical sample, colorless needles, m.p. 232–234° dec. (reported[8] 218–219°; our earlier figure[21] was a typographical error); ν_{max}^{KBr} 3480, 3125, 1672, 1630, 1603, 1542 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.09; H, 5.89; N, 29.22. Found: C, 50.24; H, 6.04; N, 28.96.

An analytical sample of **triacanthine picrate** was formed directly (without recrystallization) from an ethanolic solution of triacanthine and ethanol saturated with picric acid; m.p. 246° dec. (reported[8] 239–241°).

Anal. Calcd. for $C_{16}H_{16}N_8O_7$: C, 44.41; H, 3.74; N, 25.94. Found: C, 44.48; H, 3.99; N, 25.91.

Triacanthine Methiodide.—A solution of 100 mg. of triacanthine, 4 ml. of methanol and 4 ml. of methyl iodide was refluxed for 24 hours. After cooling, the solution was partially concentrated and 45 mg. of crystalline solid was collected by filtration. Paper chromatography showed that this solid consisted of two components (R_f values of 0.53 and 0.69) and that the mother liquors also consisted of two components (R_f values of 0.75 [triacanthine] and 0.69). Three recrystallizations of the solid from aqueous ethanol yielded an analytical sample (which showed one spot on paper chromatography, R_f value 0.69), m.p. 227–229° dec. (reported[8] 199–203°), λ_{max}^{water} (pH 1) 277 (15,450) and 226 mμ (ε 19,100). Upon basification the solution showed λ_{max}^{water} (pH 11) 278 (14,290) and 225 mμ (15,460).

Anal. Calcd. for $C_{11}H_{16}IN_5$: C, 38.27; H, 4.67; N, 20.29. Found: C, 38.41; H, 4.60; N, 20.14.

Dihydrotriacanthine (**6-Amino-3-isopentylpurine**) (VIII, **R** = $(CH_3)_2CHCH_2CH_2$).—A mixture of 125 mg. of triacanthine, 45.6 mg. of platinum oxide and 40 ml. of glacial acetic acid was hydrogenated for 2 days at a pressure of 3 atm. at room temperature. After this time, an additional 45.6 mg. of platinum oxide was added, and the mixture was shaken for an additional 3 days under the same conditions. The resultant mixture was filtered, diluted with water and concentrated to a small volume at room temperature *in vacuo*. The residue was dissolved in water, basified with 20% potassium hydroxide solution and extracted four times with chloroform.

Paper chromatography of the aqueous layer showed a single spot with an R_f value identical with that of adenine. In order to show that the adenine arose *via* hydrogenolysis and not *via* acid cleavage, 3 mg. of triacanthine was stirred in glacial acetic acid at room temperature. At the end of this time, paper chromatography indicated that no adenine had been formed and that only triacanthine was present.

The combined chloroform extracts were dried over magnesium sulfate, filtered and concentrated to give 51.8 mg. (42%) of dihydrotriacanthine. Sublimation of this material at 135° (0.25 mm.) yielded an analytical sample, m.p. 230–231° (when mixed with triacanthine, m.p. 205–207°), pK_a' (50% DMF) 5.2; λ_{max}^{EtOH} (neutral) 273 (12,700) and (pH 1) 277 mμ (ε 18,700); λ_{min}^{EtOH} (neutral) 247 and (pH 1) 239 mμ.

Anal. Calcd. for $C_{10}H_{15}N_5$: C, 58.51; H, 7.37. Found: C, 58.55; H, 7.17.

Attempts to prepare dihydrotriacanthine at lower pressures or using shorter reaction times yielded only mixtures of triacanthine and dihydrotriacanthine. Although it was not possible to distinguish between these two compounds by paper chromatography, n.m.r. spectroscopy provided a sensitive test for purity due to the great differences in their respective spectra. Dihydrotriacanthine was heated in concentrated hydrochloric acid for 8 hours. Paper chromatography demonstrated that no reaction had occurred (under the identical conditions, adenine was formed from triacanthine).

N-Benzyltriacanthine (**6-Benzylamino-3-(γ,γ-dimethylallyl)-purine**).—N-Benzyltriacanthine was prepared by the exchange amination procedure of Whitehead and Traverso.[9] A mixture of 182 mg. (0.9 mmole) of triacanthine, 193 mg. (1.36 mmoles) of benzylamine hydrochloride and 451 mg. (4.15 mmoles) of benzylamine was heated at 180° for 18 hr. in a sealed tube. After cooling, the tube was opened, the mixture diluted with aqueous ammonia, and the aqueous layer extracted four times with chloroform. The chloroform extracts were dried over magnesium sulfate, filtered and concentrated. The resulting red oil was dissolved in a small amount of ethyl acetate and diluted with cyclohexane. Crystals formed upon standing overnight in the ice-box. Filtration yielded 120 mg. of a gray solid (41%), m.p. 143–147°. Paper chromatography showed only one spot, R_f value 0.90. One recrystallization from ethyl acetate (after decolorization with charcoal) yielded colorless needles, m.p. 150°, pK_a' (water) 5.94; λ_{max}^{EtOH} (neutral) 292 (17,100) and (pH 1) 287 mμ (ε 24,300); λ_{min}^{EtOH} (neutral) 250 and (pH 1) 244 mμ; ν_{max}^{KBr} 1625 and 1532 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{19}N_5$: C, 69.60; H, 6.53; N, 23.88; mol. wt., 293.36. Found: C, 69.34; H, 6.52; N, 24.06; mol. wt., 310 ± 10 (titrimetric).

Degradation of Triacanthine to Adenine (I) Hydrochloride.—Triacanthine (87.7 mg.) was added to concentrated aqueous hydrochloric acid. The resulting solution was heated on a steam-bath for 12 hours. The solution was then concentrated to dryness at room temperature *in vacuo*. The tan solid residue was washed repeatedly with cold ethanol to yield 47.5 mg. (56%) of a colorless solid. Paper chromatography showed a single spot, R_f value 0.21. One recrystallization from ethanol gave an analytical sample of adenine hydrochloride hemihydrate, m.p. 283–284° (reported[54] 285–286°); λ_{max}^{water} (pH 1) 262 (log ε 4.11) and (pH 13) 268 mμ (log ε 4.06) (reported λ_{max}^{water} (pH 1) 262 (log ε 4.12) and (pH 13) 267 mμ (log ε 4.08).

Anal. Calcd. for $C_5H_6ClN_5 \cdot {}^1/_2\ H_2O$: C, 33.25; H, 3.91; N, 38.79. Found: C, 33.38, 33.09; H, 4.01, 3.95; N, 38.62.

Adenine picrate was prepared from a portion of the hydrochloride by dissolving it in water and adding a saturated solution of picric acid in water. After one recrystallization from water, a yellow solid was obtained, m.p. 291° (reported[54] 290°).

When a small amount of triacanthine was heated briefly with a 50% aqueous sulfuric acid solution and then distilled in a Hickman still, it was possible to show that the distillate possessed a vapor phase chromatography peak identical in retention time to that of γ,γ-dimethylallyl alcohol.

Alkylation of Adenine with 1-Bromo-3-methyl-2-butene (**γ,γ-Dimethylallyl Bromide**).—Reduction of 3,3-dimethylacrylic acid (Aldrich Chemical Co.) with lithium aluminum hydride according to the procedure of Knights and Waight[54] gave γ,γ-dimethylallyl alcohol. An n.m.r. spectrum (CDCl_3) of this alcohol confirmed the structure which these authors had assigned to the reduction product. The alcohol was then converted to γ,γ-dimethylallyl bromide according to the procedure of Simon, Kaufmann and Schinz.[55,56] An n.m.r. spectrum of this halide ($CDCl_3$) showed a triplet at

(54) H. R. Bentley, K. G. Cunningham and F. S. Spring, *J. Chem. Soc.*, 2301 (1951).

(55) H. L. Simon, Ad. Kaufmann, Jr., and H. Schinz, *Helv. Chim. Acta*, **29**, 1133 (1946).

(56) W. Kuhn and H. Schinz, *ibid.*, **35**, 2008 (1952).

$\tau = 4.54\left(=C\begin{matrix}H\\ \diagup\\ \diagdown\end{matrix}\right)$, a doublet centered at $\tau = 6.05$ ($-CH_2-$) and a doublet centered at $\tau = 8.25\left(=C\begin{matrix}CH_3\\ \diagup\\ \diagdown\\ CH_3\end{matrix}\right)$. The absence of other peaks demonstrated the absence of rearranged allylic isomers.

A solution of 6.0 g. of adenine (0.0445 mole) and 1.0 g. of sodium (0.0445 g.-atom) in 100 ml. of absolute ethanol was cooled to −10°. To this was added dropwise (with continuous stirring) a solution of 5.36 g. (0.036 mole) of the freshly prepared and distilled bromide in 20 ml. of absolute ethanol. The mixture was allowed to warm to room temperature and then refluxed overnight. The mixture was concentrated to a small volume, diluted with water and extracted several times with chloroform. The combined chloroform extracts were extracted once with 5% sodium hydroxide, washed once with water and then dried over magnesium sulfate. After filtration, the chloroform was concentrated to give 3.33 g. (46%) of a colorless solid, m.p. 188–196°.

Exchange Amination of the Mixture of 6-Amino-3-(γ,γ-dimethylallyl)-purine and 6-Amino-9-(γ,γ-dimethylallyl)-purine with Benzylamine.—A sealed tube containing 500 mg. of the mixture formed by the alkylation of adenine by γ,γ-dimethylallyl bromide, 500 mg. of benzylamine hydrochloride and 1.182 g. of benzylamine was heated in a sealed tube at 180° for 24 hours. After cooling, the mixture was diluted with aqueous ammonia and extracted with chloroform. After drying, the chloroform was concentrated to give a red oil. This red oil was chromatographed on neutral alumina. Elution with benzene yielded **N-benzyltriacanthine**, m.p. 150°. Identity was established by m.p., mixed-m.p., infrared spectra, ultraviolet spectra and X-ray powder patterns.

Elution with 10% chloroform in benzene yielded a second fraction. Several recrystallizations from ethyl acetate yielded an analytical sample of 6-benzylamino-9-(γ,γ-dimethylallyl)-purine (Xb), m.p. 163.3–163.8°; λ_{max}^{EtOH} (neutral) 278 (15,780) and (*p*H 1) 285 mμ (ε 20,600); λ_{max}^{EtOH} (neutral) 239 and (*p*H 1) 245 mμ; ν_{max}^{KBr} 1600 and 1550 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{19}N_5$: C, 69.60; H, 6.53. Found: C, 69.87; H, 6.80.

Attempted Conversion of N-Benzyltriacanthine to Triacanthine.—A mixture of 105.7 mg. of N-benzyltriacanthine, 49.1 mg. of ammonium chloride and 20 ml. of liquid ammonia was sealed in stainless steel bomb and heated at 170° for 24 hours. After cooling, the mixture was examined by means of paper chromatography for the presence of triacanthine. In this manner it was shown that there had been no triacanthine formed. Variations of these conditions were equally unsuccessful.

Attempts to hydrogenolyze the N-benzyl group showed that, despite wide variations of catalyst, solvent, pressure and temperature, hydrogenolysis of the allyl group took place (and probably also some hydrogenation of the double bond) at a rate comparable to debenzylation.

Direct Separation of the Mixture of 6-Amino-3-(γ,γ-dimethylallyl)-purine and 6-Amino-9-(γ,γ-dimethylallyl)-purine.—The crude alkylation product was divided into two fractions by means of a single recrystallization. These two fractions were then chromatographed separately on a column packed with deactivated alumina. For each gram of mixture placed on the column, 750 g. of alumina was employed.

Elution with 10% ethanol in chloroform yielded **6-amino-9-(γ,γ-dimethylallyl)-purine (Xa)**, m.p. 167–168°. Sublimation at 160° (0.01 mm.) gave an analytical sample, m.p. 167–168°, pK_a' (50% DMF) 3.3; λ_{max}^{EtOH} (neutral) 260 (13,800) and (*p*H 1) 260 mμ (ε 14,000); λ_{min}^{EtOH} (neutral) 228 and (*p*H 1) 232 mμ; ν_{max}^{KBr} 1660, 1585 and 1570 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45. Found: C, 59.30; H, 6.45.

The hydrochloride was prepared by dissolving the free base in ethanol and saturating this cold solution with anhydrous hydrochloric acid at 0°. The crystalline solid formed immediately. After two recrystallizations from ethanol, an analytical sample (fine, colorless needles) was obtained, m.p. 247–248° dec. (gas evolution, odor of isoprene); ν_{max}^{KBr} 2600, 1710, 1645 and 1613 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.09; H, 5.89; N, 29.22. Found: C, 50.35; H, 5.92; N, 29.15.

Further elution of the column yielded no more material until 25% ethanol in chloroform was used as an eluent. Material which was eluted with this solvent system was pure **synthetic triacanthine**, m.p. 228–229°. Identification was further established *via* mixed m.p., infrared spectrum and ultraviolet spectra. A hydrochloride prepared from this synthetic triacanthine was identical (by mixed m.p.) with authentic triacanthine hydrochloride. The triacanthine which was eluted from the column corresponded to 65% of the total material isolated from the column. Only 60% of the material which was originally applied to the column was recovered. From an ultraviolet spectrum of the original alkylation mixture, it was possible to determine that the percentage of triacanthine in this mixture was 70%.

6-Amino-7-(γ,γ-dimethylallyl)-purine (IV).—A solution of 2.5 g. (0.0357 mole) of γ,γ-dimethylallyl alcohol in 50 ml. of absolute ether was cooled to −70°. To this was added (over a period of 4 hours) a solution of 2.4 ml. (0.0825 equiv.) of phosphorus trichloride in 100 ml. of ether. To this ether solution at −70° (containing γ,γ-dimethylallyl chloride), was added 5.0 g. (0.0357 mole) of adenine and 4.9 g. (0.214 g.-atom) of sodium which had been dissolved in 150 ml. of dry ethanol. After addition was complete (30 minutes), the mixture was allowed to warm to room temperature and then heated at reflux for 6 hours. The solution was cooled and concentrated to a small volume. The residue was diluted with water and the basic solution extracted with chloroform. The combined extracts were dried over magnesium sulfate, filtered and concentrated to give 230 mg. of solid (3%). Further purification was effected by distribution chromatography on cellulose (using the solvent system which was employed in the paper strip chromatographic separations). Combination of appropriate fractions (as indicated by paper chromatography) gave 125 mg. of colorless solid. Sublimation at 160° (0.1 mm.) yielded an analytical sample, m.p. 195–196°, pK_a' (50% DMF) 3.2; λ_{max}^{EtOH} (neutral) 272 (9,750) and (*p*H 1) 276 mμ (ε 14,600); λ_{min}^{EtOH} (neutral) 231 and (*p*H 1) 242 mμ; ν_{max}^{Nujol} 3420, 3330, 1625, 1605 and 1545 cm.$^{-1}$. An n.m.r. spectrum (20% in liquid sulfur dioxide) was almost identical with that of triacanthine.

Peak	Area	Assignment
1.81	1	Ring H
1.95	1	Ring H
4.49	1	C=C—H
5.01	2	N—CH_2—C=
8.13	6	C=C—CH_3

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45. Found: C, 58.89; H, 6.40.

The hydrochloride was prepared in the usual manner, m.p. 203–205° dec.; ν_{max}^{Nujol} 3420, 3200, 1660, 1620 and 1590 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.09; H, 5.89. Found: C, 50.29; H, 6.00.

6-Amino-7-(*cis*-β-,γ-dimethylallyl)-purine (XII).—*cis*-2-Methyl-2-buten-1-ol was prepared by the method of Hatch and Noyes[57] *via* the lithium aluminum hydride reduction of tiglic acid.

A solution containing 2.5 g. (0.0357 mole) of the alcohol in 10 ml. of dry ether was cooled to −70°. A solution of 2.4 ml. (0.0825 equiv.) of phosphorus trichloride in 24 ml. of dry ether was then added to the ether solution of the alcohol over a period of 4 hours. The solution was allowed to warm to room temperature. This solution, containing the *cis*-1-chloro-2-methyl-2-butene, was used immediately in the next step. A solution of 5.0 g. (0.0371 mole) of adenine, 4.95 g. (0.217 g.-atom) of sodium and 30 ml. of absolute ethanol was added to the stirred solution of the chloro compound (at −70°). The resulting mixture was allowed to warm to room temperature and was then heated under reflux overnight. After cooling, the solution was partially concentrated, diluted with water, and the strongly basic solution was extracted with chloroform. After drying, the combined chloroform extracts were evaporated to dryness to yield 712 mg. (approximately 16% over-all yield) of a tan solid. Further purification was accomplished *via* distribution chromatography. Sublimation of the purified material at 160° (0.1 mm.) yielded an analytical sample, m.p. 250°,

(57) L. F. Hatch and P. R. Noyes, *J. Am. Chem. Soc.*, **79**, 345 (1957).

pK_a' (50% DMF) 3.2; λ_{max}^{EtOH} (neutral) 272 (9,200) and (pH 1) 276 mμ (ε 14,400); λ_{min}^{EtOH} (neutral) 233 and (pH 1) 242 mμ; ν_{max}^{Nujol} 3460, 3374, 1672 and 1625 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45. Found: C, 58.95; H, 6.46.

It was also shown that it was possible to cleave this material to adenine under the same conditions employed for the similar cleavage of triacanthine.

A hydrochloride was formed in the usual manner, m.p. 216–218° dec.; n.m.r. spectrum (10% in D_2O) showed the following peaks:

Peak	Area	Assignment
1.33 (doublet)	2	Ring H's
4.34 (quartet)	1	C=C—H
4.82 (singlet)	2	N—CH_2—C=
8.22 (doublet and singlet)	6	C=C—CH_3

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.09; H, 5.89. Found: C, 49.78; H, 5.81.

6-Amino-7-(*trans*-β,γ-dimethylallyl)-purine (XIV).—Angelic acid, prepared from tiglic acid by the method of Buckles and Mock,[58] was reduced with lithium aluminum hydride to *trans*-2-methyl-2-buten-1-ol. A solution containing 1.95 g. of the alcohol (0.0279 mole) in 50 ml. of absolute ether was cooled to −70°. Over a period of 4 hours, 1.62 ml. (0.0558 equiv.) of phosphorus trichloride in 100 ml. absolute ether was added to the alcohol solution. The solution was stirred for an additional half-hour and then (containing the *trans*-1-chloro-2-methyl-2-butene) was used immediately in the next step. A solution of 3.76 g. (0.028 mole) of adenine and 2.0 g. (0.087 g.-atom) of sodium in ethanol was added to the solution of the chloro compound over a period of 40 minutes at −70°. The temperature was allowed to rise to about 25°, and the solution was then heated under reflux for 8 hours. The mixture was concentrated to a small volume, and the strongly basic solution was extracted with chloroform. After drying the chloroform extracts over magnesium sulfate, the chloroform was filtered and concentrated to a small volume to yield 104 mg. (approximately 7% over-all yield). Further purification was accomplished *via* distribution chromatography on cellulose. Sublimation of the purified material at 160° (0.1 mm.) yielded an analytical sample, m.p. 214°; λ_{max}^{EtOH} (neutral) 272 (9,200) and (pH 1) 277 mμ (ε 14,400); λ_{min}^{EtOH} (neutral) 234 and (pH 1) 242 mμ; ν_{max}^{Nujol} 1670 and 1620 cm.$^{-1}$ in the 6μ region (similar to the *cis* isomer); n.m.r. spectrum (10% in D_2O), as the hydrochloride, showed the following peaks:

Peak	Area	Assignment
1.37 (doublet)	2	Ring H's
3.94 (quartet)	1	C=C—H
4.68 (singlet)	2	N—CH_2—C=C
8.14 (doublet and singlet)	6	C=C—CH_3

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45. Found: C, 59.00; H, 6.48.

Alkylation of Adenine with Bromoacetaldehyde Diethyl Acetal.—A mixture of 5.0 g. of adenine (0.037 mole), 0.85 g. of sodium (0.037 g.-atom) and 6.0 g. of redistilled bromoacetaldehyde diethyl acetal[34] (0.0305 mole) in 100 ml. of absolute ethanol was refluxed for 4 days. The resultant solution was then concentrated to a small volume under reduced pressure and diluted with water. This basic solution was extracted four times with chloroform. After drying over magnesium sulfate the combined chloroform extracts were filtered and concentrated to give a paste. Dilution of the paste with ether and filtration yielded 3.1 g. (33%) of a colorless solid. A portion of the crude product was chromatographed on neutral alumina (using a large excess). By suitable combination of fractions (on the basis of their paper chromatographic characteristics), two pure components were obtained:

6-Amino-9-(β,β-diethoxyethyl)-purine (XV).—The first fractions to be eluted from the column (5% ethanol in chloroform) were recrystallized from ethanol to yield a colorless, crystalline solid, m.p. 217–218°; λ_{max}^{EtOH} (neutral) 260 (14,700) and (pH 1) 259 mμ (ε 14,570); λ_{min}^{EtOH} (neutral) 227 and (pH 1) 232 mμ; ν_{min}^{KBr} 1660, 1600 and 1570 cm.$^{-1}$.

(58) R. E. Buckles and G. V. Mock, *J. Org. Chem.*, **15**, 680 (1950).

Anal. Calcd. for $C_{11}H_{17}N_5O_2$: C, 52.57; H, 6.82; N, 27.87. Found: C, 52.81; H, 6.77; N, 27.60.

6-Amino-7-(β,β-diethoxyethyl)-purine (XVI).—The second component to be isolated in pure form from the chromatography was recrystallized from ethanol–petroleum ether to yield colorless prisms, m.p. 166–168°, pK_a' 3.3; λ_{max}^{EtOH} (neutral) 271 (9,350) and (pH 1) 275 mμ (ε 15,350); λ_{min}^{EtOH} (neutral) 239 and (pH 1) 242 mμ; ν_{max}^{KBr} 1651, 1610 and 1565 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{17}N_5O_2$: C, 52.57; H, 6.82. Found: C, 52.83; H, 6.82.

The structure assignment is here based on the dissociation constant and on the ultraviolet spectral shifts encountered (maxima and minima) in going from neutral to acidic solution.

Hydrolysis of 6-Amino-9-(β,β-diethoxyethyl)-purine (XV). (1).—A solution of 268 mg. of the acetal in 1 *N* hydrochloric acid was heated for 7 minutes at 80° and was then allowed to stand overnight. Partial concentration of the solvent yielded crystalline material which was recrystallized from ethanol–petroleum ether as fine, colorless needles, m.p. 309–311° dec., yield 196 mg.; λ_{max}^{EtOH} (pH 1) 257 (15,400); (neutral salt) 259 (15,100); and (pH 13) 259 mμ (ε 15,600); λ_{max}^{EtOH} (pH 1) 232; neutral salt) 233; pH 13) 235 mμ.

Anal. Calcd. for $C_9H_{14}ClN_5O_2$: C, 41.62; H, 5.43; N, 26.97; O, 12.32. Found: C, 41.63; H, 5.47; N, 27.05; O, 12.48.

On the basis of the analysis and ultraviolet spectra, this compound was tentatively assigned the hemiacetal structure, **6-amino-9-(β-ethoxy-β-hydroxyethyl)-purine hydrochloride (XIX). (2).**—A mixture of 54 mg. of the acetal XV, 4 ml. of glacial acetic acid, 20 ml. of water and 1.5 g. of zinc was heated under reflux for 1 hour. The mixture was distilled, and the first drops to come over were collected in a 2,4-dinitrophenylhydrazine solution. Filtration of the solid 2,4-dinitrophenylhydrazone derivative yielded 6.6 mg. (15%) of acetaldehyde 2,4-dinitrophenylhydrazone. This identification is based on infrared spectral data.

(3).—Attempted isolation of the aldehyde under conditions more vigorous than those employed in the isolation of the hemiacetal were unsuccessful. In addition to starting material, water-soluble components were obtained which could not be purified and which exhibited reversible color changes with varying pH.

Pyrolysis of Triacanthine Hydrochloride—Isolation of the Non-volatile Components—Adenine Hydrochloride and Pyrotriacanthine Chloride.—A sealed tube containing 133.6 mg. of triacanthine hydrochloride was heated at 280° for 30 minutes. The tube was cooled and opened. A gas escaped which had a strong odor suggestive of isoprene. Paper chromatography showed that two components were present, neither of which was triacanthine. One component was strongly Dragendorff-positive, the other Dragendorff-negative. Both components had strong ultraviolet absorption. The pyrolysis mixture, as a brown solid, was taken up in aqueous ethanol, the solution was evaporated to dryness, and the residue was extracted several times with warm ethanol. Paper chromatography showed that the ethanol-insoluble material was adenine. The ethanol-soluble fraction was evaporated to dryness; yield 94.6 mg. of solid. Paper chromatography showed that this fraction consisted of the intensely Dragendorff-positive spot and a small amount of adenine. Two recrystallizations from ethanol were sufficient to remove the remaining adenine hydrochloride and yield an analytical sample of colorless, very fine needles of pyrotriacanthine chloride (XXV), m.p. 326° dec. (with evolution of a gas with an isoprene odor); λ_{max}^{EtOH} 275 mμ (ε 11,500); ν_{max}^{Nujol} 3400, 3350, 1610 and 1590 cm.$^{-1}$; n.m.r. spectrum (liquid sulfur dioxide) had the following signals:

Peak	Area	Assignment
1.44 (singlet)	1	Ring H
1.55 (singlet)	1	Ring H
5.31 (triplet)	2	–CH_2–
5.90 (broad)		Water
7.52 (triplet)	2	–CH_2–
8.21 (singlet)	6	CH_3

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.09; H, 5.89. Found: C, 49.97; H, 5.55.

Refluxing triacanthine in ethanol which had been saturated with anhydrous hydrochloric acid also yielded pyrotriacanthine (identification made by means of paper chromatography). Under these conditions, however, a lower ratio of pyrotriacanthine to adenine was observed upon paper chromatographic inspection of the crude product.

1-Methyl-4,4,5,5-tetracyano-1-cyclohexene (XXIV).—The synthesis of authentic material was modeled after the method which would be necessary to trap isoprene from the heating of adenine hydrochloride, and the simple apparatus used was a flask, equipped with a nitrogen inlet tube, from which a sealed outlet tube extended in the form of a wide, inverted U. The flask contained 95.2 mg. (0.78 mmole) of 2,5-dihydro-2-methylthiophene-1,1-dioxide (isoprene sulfone) and the end of the inverted U-tube extended below the surface of a solution of 102.4 mg. (0.81 mmole) of tetracyanoethylene[59] in 2 ml. of anhydrous tetrahydrofuran in a test-tube. The flask was heated to 120° in a slow stream of nitrogen until gas evolution of the molten isoprene sulfone had ceased. Evaporation of the tetrahydrofuran and drying of the residue under a high vacuum yielded 76.1 mg. (50%) of colorless needles, m.p. 95–97°. One recrystallization from ethanol yielded an analytical sample, m.p. 106–107°.

Anal. Calcd. for $C_{11}H_8N_4$: C, 67.33; H, 4.11. Found: C, 67.44; H, 3.94.

Pyrolysis of Triacanthine Hydrochloride—Isolation of the Volatile Component.—Using the same apparatus as described above, 47.9 mg. of triacanthine hydrochloride was placed in the flask. After heating the flask at 240° in a slow stream of nitrogen until gas evolution had ceased, the product was isolated as above, yielding 7.6 mg. (20%) of the adduct, m.p. 96–97°, improved on recrystallization. This material failed to depress the m.p. of the authentic sample of 1-methyl-4,4,5,5-tetracyano-1-cyclohexene.

Ozonolysis Studies. (1) Ozonolysis of N-Benzyltriacanthine.—Ozone (generated by a Wilzbach ozone generator) was passed through a solution of 86 mg. (0.296 mmole) of N-benzyltriacanthine in 15 ml. of 20% acetic acid–80% acetic anhydride at −70° at a concentration of 1 mmole per cc. of gas. The exit gas was passed through a potassium iodide solution which turned red after 5 minutes, indicating that ozone was no longer reacting with the N-benzyltriacanthine. A total of 300 ml. (0.30 mmole) had been passed through the solution during this time. The solution was then warmed to room temperature and added to 1.5 g. of zinc dust in 50 ml. of water. After 1 hour of stirring at room temperature, the mixture was distilled directly into a 2,4-dinitrophenylhydrazine solution. The solid was collected by filtration to yield 37 mg. (55%), m.p. 111–118°.

An infrared spectrum of this material (2% in chloroform) was identical with that of authentic acetaldehyde 2,4-dinitrophenylhydrazone. Authentic acetaldehyde 2,4-dinitrophenylhydrazone, m.p. 158–160°, has a characteristic infrared maximum at 1087 cm.$^{-1}$. Acetone 2,4-dinitrophenylhydrazone, m.p. 124–125°, has a characteristic maximum at 1104 cm.$^{-1}$. Spectra were determined on known mixtures of these two derivatives. By utilization of these two characteristic maxima, amounts greater than (approximately) 15% of one derivative in the other could be readily detected. The derivative (crude) from the ozonolysis of N-benzyltriacanthine, therefore, contained less than 15% of the acetone 2,4-dinitrophenylhydrazone.

Chromatography of the crude material on acid-washed alumina using 1:9 ether–petroleum ether yielded one major band and two slow-moving trace bands (not isolated). Isolation of the major band yielded, after one crystallization from ethanol, a product of m.p. 158–160°, undepressed in m.p. on admixture with authentic acetaldehyde 2,4-dinitrophenylhydrazone.

Similar ozonization of triacanthine (using acetic acid–acetic anhydride as a solvent at room temperature) also yielded the 2,4-dinitrophenylhydrazone of acetaldehyde (identification by means of infrared spectrum) in somewhat lower yield.

(2) Ozonolysis of the Product Obtained from the Alkylation of Adenine with *cis*-1-Chloro-2-methyl-2-butene.—Ozonization of this material in acetic acid–acetic anhydride mixture of 0° yielded, after reductive work-up with zinc, 110 mg. of a 2,4-dinitrophenylhydrazone derivative. An infrared spectrum of this material was identical with that of authentic acetone 2,4-dinitrophenylhydrazone except for a shoulder at 1087 cm.$^{-1}$ (indicative of approximately 15% of the 2,4-dinitrophenylhydrazone of acetaldehyde). Chromatography (using the method described for acetaldehyde 2,4-dinitrophenylhydrazone) yielded crystalline material, m.p. 124–125°, undepressed upon mixture with authentic acetone 2,4-dinitrophenylhydrazone.

(3) Ozonolysis of Triacanthine in Methanol.—A solution of 64 mg. of triacanthine in 10 ml. of absolute methanol was ozonized at 0°. After uptake of the ozone had ceased, the solution was added to a mixture of 70 ml. of water, 10 ml. of acetic acid and 1.5 g. of zinc. Isolation of the low molecular weight carbonyl components *via* steam distillation as the 2,4-dinitrophenylhydrazones yielded 43 mg. (58%) of acetone 2,4-dinitrophenylhydrazone. It was also possible to obtain by the partial concentration of the mother liquors 14 mg. of the more soluble acetaldehyde 2,4-dinitrophenylhydrazone (containing some of the acetone 2,4-dinitrophenylhydrazone). The preceding identifications were made by means of inspection of the infrared spectra.

(4) Ozonolysis of Triacanthine in Methanol–Acetic Acid.—In a manner similar to the preceding experiment, 50 mg. of triacanthine was ozonized at 0° in a solution of 2 ml. of glacial acetic acid and 8 ml. of methanol. Isolation of the products as the 2,4-dinitrophenylhydrazones yielded 15.4 mg. (31%) of acetone 2,4-dinitrophenylhydrazone containing a small amount of acetaldehyde 2,4-dinitrophenylhydrazone.

(59) W. J. Middleton, R. E. Heckert, E. L. Little and C. G. Krespan, *J. Am. Chem. Soc.*, **80**, 2783 (1958).