04-4; **9**, 21343-05-5; **10**, 21343-06-6; **11**, 21343-08-8; **13**, 5400-01-1.

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Aminoacyl Nucleosides. V. The Mechanism of the Rearrangement of N^6-(α-Aminoacyl)adenines into N-(6-purinyl)amino Acids[1]

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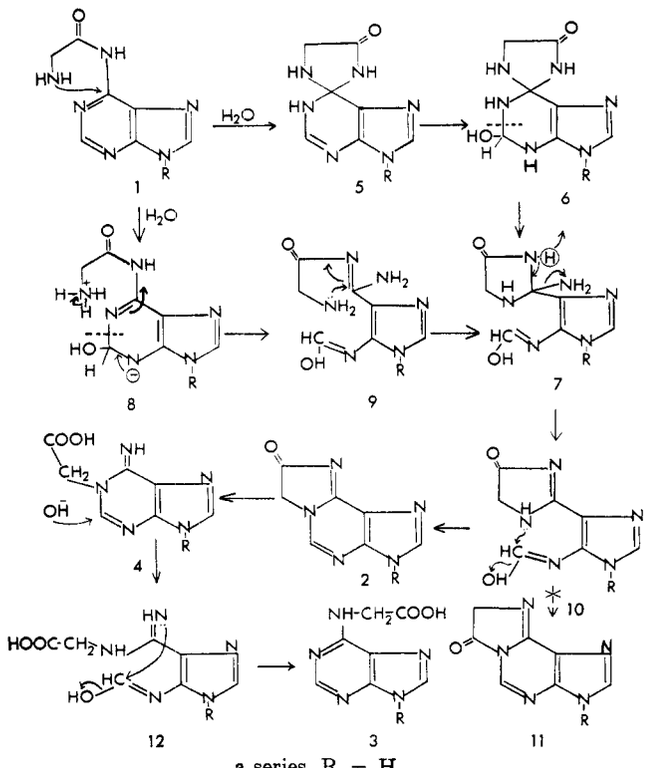
N^6-(Glycyl-α-^{15}N)adenine (**1**), on standing in water at neutral pH, loses the elements of ammonia to yield the cyclic intermediate 3*H*-imidazo[2,1-*i*]purin-8(7*H*)-one (**2**) with complete retention of the ^{15}N. Compound **2**, on warming in water, rearranges to form N-(6-purinyl)glycine (**3**) through an intermediate, 1-carboxymethyladenine (**4**). The same reaction was carried out on N^6-sarcosyladenine-6-^{15}N (**15**). A single product, N-(6-purinyl)sarcosine (**16**), containing 100% of the atom excess ^{15}N, was obtained. On the basis of these results, a mechanism for the rearrangement of N^6-(α-aminoacyl)adenines to N-(6-purinyl)amino acids is proposed. The α-amino group of **1** adds across the C^6–N^1 double bond, and the pyrimidine ring opens. The N^1 of the purine is expelled as ammonia, and the pyrimidine ring re-forms to yield **2**. After lactam opening, **2** behaves as an N^1-alkyladenine, and undergoes the well-known $N^1 \rightarrow N^6$ rearrangement to form **3**.

This paper reports a further study of a rearrangement in which N^6-glycyladenine, **1a**, is converted into N-(6-purinyl)glycine, **3a** (Chart I), with elimination of the elements of ammonia.[2] The rearrangement is general for N^6-(α-aminoacyl)adenines.[4] In addition, N^6-glycyl-9-methyladenine, **1b**, undergoes a similar conversion, which indicates that N^9 hydrogen of purine is not essential for the rearrangement.[1] The studies with **1b** also indicate that the rearrangement could occur at a nucleoside level. The blocked aminoacyladenines like N^6-(N-benzyloxycarbonylglycyl)adenine and N^6-(N-formylglycyl)adenine, when heated in water, do not undergo this rearrangement, which suggests that the presence of a free amino nitrogen is necessary. N^6-(N,N-Dimethylglycyl)adenine and N^6-[α-N-(piperidyl)acetyl]adenine[5] undergo hydrolysis to adenine when subjected to the rearrangement conditions. There appears to be no direct involvement of the imidazole portion of a purine molecule in this rearrangement.

CHART I



a series, R = H
b series, R = CH_3

The first step in the reaction for both N^6-glycyladenine, **1a**, and N^6-glycyl-9-methyladenine, **1b**, involves the elimination of ammonia and the formation of the isolable intermediates, **2a** and **2b**, respectively (Chart I). The structure of **2a** was previously established by comparing its properties with those of a sample synthesized from N^6-chloroacetyladenine.[2] In the latter case, **2a** results from nucleophilic displacement of the chloride by the N^1 nitrogen of N^6-chloroacetyladenine. It is obvious that **2a** could not result (from **1a**) from an analogous intramolecular displacement. The α-amino group must participate in the reaction, because under identical conditions, N^6-acetyladenine undergoes simple hydrolysis to yield adenine.

In order to explain this rearrangement, we had earlier proposed a mechanism based on an addition–elimination reaction, suggesting loss of the N^1 nitrogen of adenine.[2] We now wish to consider this mechanism in more detail and to offer experimental evidence in support of the proposed mechanism. In the initial

(1) Paper IV: G. B. Chheda and R. H. Hall, *J. Org. Chem.*, **34**, 3492 (1969).
(2) G. B. Chheda and R. H. Hall, *Biochemistry*, **5**, 2082 (1966).[3]
(3) Paper III in the series.
(4) G. B. Chheda and R. H. Hall, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., p 061, 1966.
(5) F. Craveri and G. Zoni, *Chimica* (Milan), **34**, 407 (1958).

step of this mechanism, the α-amino group of the aminoacyl moiety of **1** (Chart I) adds across the C^6–N^1 bond of adenine to form the possible transient intermediate **5**. Saturation of the C^6–N^1 bond is known to labilize the C^2–N^1 bond in purine nucleus;[6] thus, the intermediate **5** could undergo ring opening *via* the hydrated intermediate **6** to give **7**. An alternative mechanism for the formation of intermediate **7** would involve the addition of water to **1** by intramolecular base catalysis to give intermediate **8**, with a C^2–N^3 saturated bond. In compound **8**, the α-amino group as the conjugate acid should facilitate the ring cleavage to give an intermediate **9**, which in turn could undergo addition of the amino group across the C^6–N^6 bond to yield the same intermediate **7**. Formation of **7** from the assumed cyclic intermediate **5** seems more likely than the alternative route of **8** → **9** → **7** (Chart I).

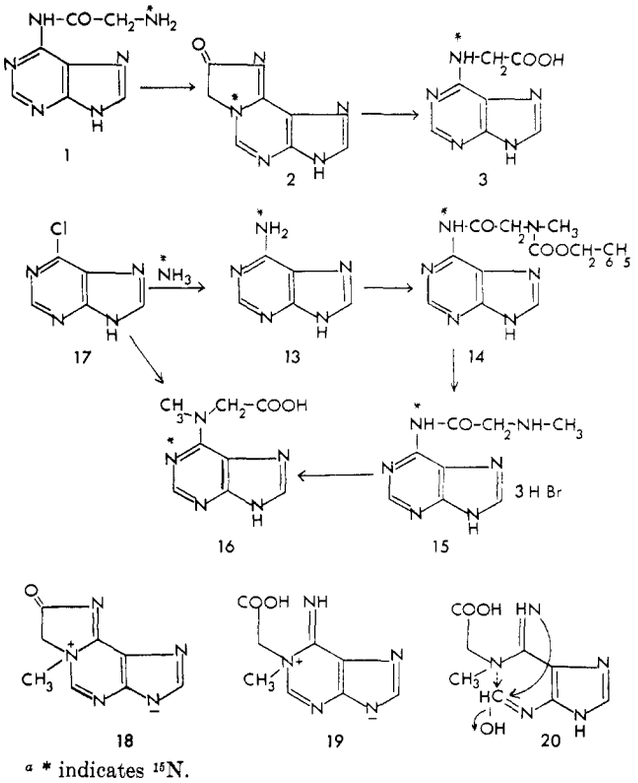
Intermediate **7**, in either case, should readily undergo elimination of ammonia to yield a transient intermediate **10**. The C^2 of **10** would be attacked by the more basic amino nitrogen originating from the amino acid, rather than by the amino nitrogen originating from the N^6 of adenine. Accordingly, the cyclic product obtained should have the structure **2** rather than the alternative structure **11**.[7] Conversion of compound **2a** into the final product, *N*-(6-purinyl)glycine, **3a**, would occur by hydrolysis of the lactam to give the isolable 1-carboxymethyladenine, **4a**, followed by a typical N^1 → N^6 rearrangement[8–10] through an intermediate, **12a**. This rearrangement occurs slowly in water between pH 6 and 7, and to a lesser extent in acidic solution, but proceeds rapidly in basic solution.

The formation of *N*-(6-purinyl)glycine, **3a**, might occur by a third route directly from **5a** by simple hydrolysis of the lactam, followed by the elimination of ammonia. This mechanism, however, does not account for the formation of the isolable cyclic compound **2a** and 1-carboxymethyladenine, **4a**.

In order to establish whether this general picture of the mechanism was correct, it was necessary to determine which of the three nitrogens, N^1, N^6, or the α-N of glycine, is actually expelled during the reaction. The reactions were carried out with ^{15}N-labeled compounds, N^6-(glycyl-α-^{15}N)adenine, **1**, and N^6-sarcosyladenine-6-^{15}N, **15** (Chart II).

Compound **1** was prepared by the following sequence of reactions (Chart II). Glycine-α-^{15}N (20% ^{15}N) was treated with benzyl chloroformate to yield *N*-benzyloxycarbonylglycine.[11] The *p*-nitrophenyl ester of *N*-cbz-glycine[12] was prepared by treating *N*-cbz-glycine-α-^{15}N with *p*-nitrophenyl trifluoroacetate in pyridine.[12] Condensation of adenine with *N*-cbz-glycine-α-^{15}N *p*-nitrophenyl ester in a mixture of dimethylformamide and dimethyl sulfoxide gave an excellent yield of N^6-(*N*-benzyloxycarbonylglycyl-α-^{15}N)adenine. Treatment of this compound with 30% hydrogen bromide in acetic acid gave N^6-(glycyl-α-^{15}N)adenine trihydrobromide, **1**, in quantitative yield. When **1** was heated in water for 35 min at pH 5.5, the cyclic intermediate **2** was obtained in good yield. Compound **2** was identical with the compound **2a** prepared from unlabeled **1** and from N^6-chloroacetyladenine with respect to infrared and ultraviolet spectroscopy and mobilities on paper chromatography. This sample of **2** fully retained the atom excess of ^{15}N.

CHART II[a]



[a] * indicates ^{15}N.

Under the conditions that affect the lactam opening and rearrangement, **2** was converted into *N*-(6-purinyl)-glycine, **3**, which also fully retained the atom excess of ^{15}N. Comparison of the low-resolution mass spectra of *N*-(6-purinyl-6-^{14}N)glycine with the 6-^{15}N-labeled sample showed that peaks containing ^{15}N could not be attributed to fragments stemming from the purine nucleus.

These results demonstrate that the α-N of glycyladenine is retained, and hence that the expelled nitrogen must come from adenine. In order to determine which nitrogen is expelled, N^1 or N^6, and where the nitrogen from the glycyl residue migrates to, the properties of N^6-sacrosyladenine-6-^{15}N [*i.e.*, N^6-(*N*-methylglycyl)adenine-6-^{15}N], **15**, were studied.

Adenine-6-^{15}N was prepared by displacing the chlorine of 6-chloropurine with ammonia-^{15}N. Reaction with *p*-nitrophenyl trifluoroacetate in anhydrous pyridine[12] converted *N*-cbz-sarcosine into its *p*-nitrophenyl ester. Condensation of this ester with adenine, **13**, in a mixture of dimethylformamide and dimethyl sulfoxide gave an excellent yield of N^6-(*N*-benzyloxycarbonylsarcosyl)adenine-6-^{15}N, **14**. The benzyloxycarbonyl

(6) V. P. Skulachev, Abstracts, Sixth International Congress of Biochemistry, New York, N. Y., p 758, 1964.

(7) Since our initial studies of this reaction, additional evidence has been obtained that supports the structure assigned to compound **2a**. When the cyclic intermediate **2a** obtained from **1a** was allowed to stand in an excess of 0.1 *N* NaOH solution at room temperature, it gave a new compound, **4a**, whose ultraviolet spectra were identical with those of 1-methyladenine. When heated in alkali, **4a** was converted into *N*-(6-purinyl)glycine, **3a**. The structure of **4a**, then, must be 1-carboxymethyladenine. This N^1-alkylated adenine can be obtained only if the cyclic compound has the 8-oxo structure, **2a**. If the cyclic compound had the alternative structure, **11a**, the resulting product would have to be **3a**.

(8) D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1276 (1963).

(9) J. Goerdeler and W. Roth, *Chem. Ber.*, **96**, 534 (1963).

(10) E. C. Taylor and P. K. Leoffler, *J. Amer. Chem. Soc.*, **82**, 2147 (1960).

(11) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(12) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Jap.*, **37**, 1231 (1964).

group was removed by treatment with a 30% solution of hydrogen bromide in acetic acid to give the trihydrobromide of N^6-sarcosyladenine-6-^{15}N, **15**, in quantitative yield. An attempt to prepare N^6-sarcosyladenine by displacement of the chlorine of N^6-chloroacetyladenine with methylamine failed.

In aqueous solution at 100°, N^6-sarcosyladenine-6-^{15}N trihydrobromide, **15**, underwent rearrangement to N-(6-purinyl)sarcosine, **16**, in 63% yield. The product fully retained the atom excess of ^{15}N. N^6-Sarcosyladenine undergoes this conversion in a higher yield than N^6-glycyladenine. Treatment of 6-chloropurine, **17**, with sarcosine gave N-(6-purinyl)sarcosine, **16**, with a normal isotope content. The structure of the rearrangement product, N-(6-purinyl)sarcosine, **16**, was confirmed by comparison with the corresponding synthetic sample. In the conversion of **15** into **16**, intermediate products could not be detected by ultraviolet spectrophotometry or paper chromatography.

Assuming that the methyl group in N^6-sarcosyladenine does not alter the rearrangement mechanism depicted for N^6-glycyladenine, **1**, in Chart I, two conclusions can be drawn from this experiment. Since the methyl group of sarcosine in **15** becomes attached to N^6 in the final product, N-(6-purinyl)sarcosine, **16**, the nitrogen from the α-amino group in an N^6-(α-aminoacyl)adenine derivative becomes the N^6 of adenine, through rearrangement. Because the entire ^{15}N isotope was retained, the N^6 of N^6-sarcosyladenine, **15**, must relocate as the N^1 of N-(6-purinyl)sarcosine, **16**. These results agree with the suggested mechanism.

Our inability to isolate the intermediates analogous to compounds **2** and **4** in the conversion of N^6-sarcosyladenine, **15**, into N-(6-purinyl)sarcosine, **16**, may be due to the N-methyl analog of the intermediate **10** undergoing cleavage of the oxoimidazole moiety, giving rise directly to **20** without going through the cyclic compound **18** or the N^1-dialkyl compound **19**. The methyl analogs **18** and **19**, if formed, would be quaternary structures and would be too unstable to permit ready isolation. On ring closure, compound **20** can give rise to either the unstable **19** or the stable N-(6-purinyl)sarcosine, **16**.

The results of these experiments suggest that the mechanism of the rearrangement of N^6-(α-aminoacyl)-adenine to N-(6-purinyl)amino acids is an addition–elimination reaction following the course proposed in Chart I. Thus, the rearrangement of N^6-glycyladenine, **1**, into N-(6-purinyl)glycine, **3**, converts the α nitrogen of the amino acid moiety in **1** into the N^6 of the purine moiety in the rearranged compound **3** and the N^6 of **1** into the N^1 of **3**; the N^1 of **1** is lost during the formation of the cyclic intermediate **2**. The possibility of a different mechanistic pathway in the case of N^6-sarcosyladenine, compared with N^6-glycyladenine, cannot be fully eliminated, since the intermediates **18** and **19** were not isolated in the former.

A rearrangement discovered by Ueda and Fox[13] in pyrimidinyl-α-amino acids deserves mention at this point. In this rearrangement, a cytosine-N^4-propionic acid is converted into a cytosine-N^3-propionic acid by way of a cyclic intermediate. This rearrangement also occurs through ring opening between C_2 and N_3 followed by interchange between the nitrogens N^3 and N^4 during the reformation of the pyrimidine nucleus. It is of interest to note that the Ueda–Fox[13] rearrangement is the reverse of the Dimroth rearrangement[8] in terms of the nitrogen interchange.

(13) T. Ueda and J. J. Fox, *J. Org. Chem.*, **29**, 1762 (1964).

Experimental Section

General.—Melting points were determined in capillary tubes on a Mel-Temp melting point apparatus and are corrected. Infrared spectra were determined in KBr disks with a Perkin-Elmer 137B "Infracord" spectrophotometer. Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer.

Paper Chromatography.—The solvent systems A, B, C, D, E, and F and methods used are described in the preceding paper.[1]

^{15}N Analysis.—^{15}N analysis was performed by Gollob Analytical Service, Inc., Berkeley Heights, N. J. The samples were processed in a modified Dumas apparatus, in which the material is oxidized with copper oxide and the nitrogen oxides are reduced with hot copper. (Heated iron was employed to reduce water to hydrogen, in order to prevent interference in the mass spectrometer.) The resulting elemental nitrogen was analyzed in a mass spectrometer, and the ratio $N^{15}/N^{14} + N^{15} \times 100$ was determined.

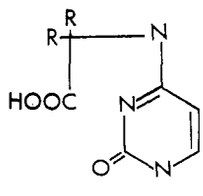
N^6-(Glycyl-α-^{15}N)adenine Trihydrobromide, 1.—Glycine (200 mg, 97% ^{15}N, Volk Radiochemical Company, Skokie, Ill.) diluted with 800 mg of glycine-^{14}N, was treated with benzyl chloroformate,[11] giving, in 75% yield, ^{15}N-benzyloxycarbonylglycine (19.4% ^{15}N), mp 118–119°. Treatment with p-nitrophenyl trifluoroacetate[12] converted ^{15}N-benzyloxycarbonylglycine into its p-nitrophenyl ester, mp 120–122°, 86% yield. Condensation of ^{15}N-benzyloxycarbonylglycine p-nitrophenyl ester (2.8 g, 8.5 mmol) with 946 mg (7.0 mmol) of adenine gave N^6-(^{15}N-benzyloxycarbonylglycyl)adenine (88% yield); mp 221–222°. The sample, recrystallized from dimethylformamide–ethanol, amounted to 1.27 g; mp 227–228° dec. This material was free of adenine and appeared as one spot in paper chromatography.

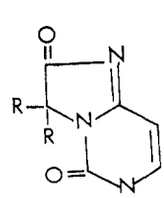
The cbz group was removed from N^6-(^{15}N-benzyloxycarbonylglycyl)adenine by treatment with a 30% solution of HBr in acetic acid, giving N^6-(glycyl-α-^{15}N) adenine trihydrobromide in 99% yield; mp 182–184° dec; uv λ_{max} pH 1, 273 and 282 mμ (shoulder); pH 5.5, 274 mμ (broad); pH 13, 279 mμ.

Anal. Calcd for $C_7H_8N_6O \cdot 3HBr$: C, 19.32; H, 2.55; N, 19.32; ^{15}N, 3.23. Found: C, 19.35; H, 2.96; N, 19.57; ^{15}N, 3.3.

3H-Imidazo[2,1-i]purin-8-(7H)-one-6-^{15}N (Cyclic Intermediate), 2.—A solution of 1.30 g (3.00 mmol) of N^6-(glycyl-α-^{15}N)-adenine trihydrobromide in 40 ml of water was adjusted to pH 5.5 by the addition of 1 N sodium hydroxide. The solution was heated in a boiling water bath for 35 min and was centrifuged to remove a colloidal purple material.[14] The desired product slowly separated as purple crystals from the clear pink solution and was

(14) The intense purple coloration of the reaction mixture[3] associated with the formation of cyclic compound **2** is probably caused by oxidation of the active methylene group of the lactam. In the preparation of the corresponding cyclic intermediates from N^6-DL-phenylalanyladenine and N^6-DL-valyladenine, there was no significant color formation. This difference appears to be due to loss of the active methylene group through replacement of one of the hydrogens by an alkyl or aryl side chain. A similar colored material was encountered by Ueda and Fox[13] in their attempts to form the cyclic compound **22** from cytosine-N^4-acetic acid, **21**. Removal of the active methylene group by substitution with methyl groups (**23**) resulted in a reaction product, **24**, in which no colored materials were detected.





21 R=H
23 R=CH_3

22 R=H
24 R=CH_3

collected on a filter and dried *in vacuo* at 100°; yield 322 mg (61.4%). The solid material did not melt, but decomposed slowly above 320°. The filtrate, after concentration and cooling, gave additional product, 88 mg; it softened at 330°, but decomposed without melting at 340°. Total yield was 410 mg (78.0%).

The first crop was recrystallized from water as a crystalline pink material which was dried *in vacuo* at 100° for 24 hr over P_2O_5; yield 140 mg; it did not melt, but decomposed above 345°. This cyclic product was identical with the product prepared from N^6-glycyladenine and from N^6-chloroacetyladenine with respect to mobility on paper chromatography and ir and uv spectra; it had uv λ_{max} pH 1, 282 mμ; pH 5.5, 303, 269, 220 mμ; pH 13, 306 mμ. Paper chromatography ($R_f \times 100$) in various solvents showed A, 25; B, 25; C, 3.2; D, streaked; E, streaked; F, 9.3. These values are in excellent agreement with those of the ^{14}N compound, **2a**.

Anal. Calcd for $C_7H_5N_5O$: C, 47.98; H, 2.87; N, 40.0; ^{15}N, 3.96. Found: C, 47.73; H, 2.95; N, 39.88; ^{15}N, 3.9.

The formation of cyclic intermediate **2** is favored over hydrolysis when the reaction is carried out at pH 4–6. When N^6-glycyladenine is heated at pH 7 or above, a considerable amount of adenine is obtained.

***N*-(6-Purinyl)glycine-α-^{15}N, 3.**—A solution of 88.0 mg (0.502 mmol) of the cyclic intermediate **2** in 6 ml of 1 *N* NaOH was refluxed for 10 hr. The reaction mixture was concentrated to about 3 ml, and the pH was adjusted to 2.5 by dropwise addition of 97% formic acid. The precipitated *N*-(6-purinyl)glycine was collected on a filter; yield 85 mg, mp >300° dec. This material was crystallized from hot water; yield 30 mg, mp 300° dec. The product was dried *in vacuo* at 100° for 48 hr over P_2O_5, and was analyzed. The paper chromatography and ultraviolet spectra were identical with those of the material prepared from 6-chloropurine and glycine; it had uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 274 mμ (15.9); pH 5.8, 268 mμ (15.9); pH 13, 274 mμ (18.2); paper chromatography ($R_f \times 100$) A, 32; B, 43; C, 1.5; D, 43; F, 33.

Anal. Calcd for $C_7H_7N_5O_2$: N, 36.26; ^{15}N, 3.96. Found: N, 36.13; ^{15}N, 3.65.

***N*-Benzyloxycarbonylsarcosine *p*-Nitrophenyl Ester.**—To a stirred solution of 2.23 g (10.0 mmol) of *N*-benzyloxycarbonylsarcosine in 3.5 ml of anhydrous pyridine at 25° was added 2.35 g (10.0 mmol) of *p*-nitrophenyl trifluoroacetate. After 200 ml of water was added, the reaction mixture was extracted with chloroform (three 20-ml portions), dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to give a pale yellow syrup, 3.40 g (99.0%). This material was quite suitable for the next step in the synthesis; it had ir (film) $\bar{\nu}_{max}$ 1800 (ester C=O), 1720 (urethane C=O), 1625, 1600, 1540, and 1500 (C=C, NO_2), 1220 (ester COC), 860, 760, and 700 cm^{-1} (phenyl).

Adenine-6-^{15}N, 13.—A solution of 618 mg (4.00 mmol) of 6-chloropurine in 12 ml of ethanol containing 7 mmol of $^{15}NH_3$ was heated in a stainless steel bomb at 100° for 51 hr. The mixture was evaporated to dryness *in vacuo*, and the residue was then triturated with 25 ml of hot water. The insoluble brown material (90 mg) was filtered, and the filtrate was concentrated to 10 ml and was allowed to crystallize at 5°. The resulting white solid was collected on a filter, and was dried *in vacuo* at 100° for 2 hr; yield 315 mg. Paper chromatography revealed that this material contained a significant amount of 6-chloropurine, and hence the sample was purified by column partition chromatography on Celite-545 with solvent C. The 6-chloropurine was eluted in the first 80 ml of solvent, and adenine was eluted in the next 125 ml. Evaporation of the second eluate to dryness gave 140 mg of adenine, which was found to be homogeneous by paper chromatography, and required no further purification. Paper chromatography ($R_f \times 100$) showed A, 45; B, 29; C, 26; D, 63; E, 27; F, 25. The R_f values are identical with those of ^{14}N-adenine.

***N*⁶-(*N*-Benzyloxycarbonylsarcosyl)adenine-6-^{15}N, 14.**[15]—To a solution of 3.40 g (10.0 mmol) of *N*-benzyloxycarbonylsarcosine *p*-nitrophenyl ester in 8 ml of *N*,*N*-dimethylformamide was added a hot solution of 675 mg (5.00 mmol) of adenine, which was 20% adenine-6-^{15}N, in a mixture of 7 ml of *N*,*N*-dimethylformamide and 7 ml of dimethyl sulfoxide. The solution was heated at 90° for 4.5 hr and evaporated *in vacuo* to a thick syrup (bath temperature 50°). The syrup was triturated with 50 ml of chloroform, and the white solid that separated out was collected on a filter and washed with chloroform (three 10-ml portions) and ether (four 10-ml portions); yield 1.27 g (75%), mp 235–237°. Recrystallization of the product from a mixture of 100 ml of *N*,*N*-dimethylformamide and 300 ml of ethanol gave a white crystalline solid, which was collected on a filter and washed with acetone (three 5-ml portions); yield 0.820 g; mp 235–237°; uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 276 and 283 mμ (13.6, 13.6); pH 5.8, 280 mμ (12.6); pH 13, 279 mμ (13.2); ir (Nujol) $\bar{\nu}_{max}$ 2800–2100 (acidic H), 1750 (urethan C=O), 1700 (amide I C=O), 1640 (sh), 1625, 1580, and 1560 cm^{-1} (C=C, C=N, and amide II). Paper chromatography ($R_f \times 100$) showed A, 82; B, tailed; D, 87; E, tailed.

Anal. Calcd for $C_{16}H_{16}N_6O_3$: C, 56.5; H, 4.74; N, 24.7. Found: C, 56.4; H, 4.90; N, 24.5.

***N*⁶-Sarcosyladenine-6-^{15}N Trihydrobromide, 15.**[15]—A suspension of 340 mg (1.0 mmol) of N^6-(*N*-benzyloxycarbonylsarcosyl)-adenine-6-^{15}N in 2 ml of 30% HBr in acetic acid was stirred magnetically for 1 hr. Ether (5 ml) was added to the mixture, and the white crystalline solid that separated out was collected on a filter and washed with ether (four 5-ml portions). The product was dried *in vacuo* at room temperature for 48 hr over sodium hydroxide pellets and then over phosphorus pentoxide at 100° *in vacuo* for 5 hr; yield 437 mg (97.4%); mp 180–181° dec; uv λ_{max} pH 1, 274 and 280 mμ; pH 7, 277 mμ; pH 13, 281 mμ; ir $\bar{\nu}_{max}$ (Nujol) 2800–2100 (acidic H), 1750, 1680, 1625, and 1570 cm^{-1} (C=C, C=N, C=O). Paper chromatography ($R_f \times 100$) showed A, 47; B, 29 (elongated spot); C, streaked; D, 61; E, 27; F, 15. R_f values are in agreement with those of the ^{14}N compound.

Anal. Calcd for $C_8H_{10}N_6O \cdot 3HBr$: C, 21.4; H, 2.92; N, 18.7; ^{15}N, 3.3 [calculated on the basis of 99.0% ^{15}N-ammonia (Volk Radiochemicals Co., Skokie, Ill.]. Found: C, 21.5; H, 3.03; N, 18.4; ^{15}N, 2.9% of total N.

***N*-(6-Purinyl)sarcosine, 16.**[16] **A. From N^6-Sarcosyladenine-6-^{15}N.**—A solution of 224.5 mg (0.50 mol) of N^6-sarcosyladenine-6-^{15}N trihydrobromide in 10 ml of water was heated at 100° for 30 min. The pH of the solution both before and after treatment was 1.3.[16] The pH was adjusted to 3.0 by the addition of 1 *N* sodium hydroxide, and the solid that separated out was collected on a filter and washed with water (5 ml) and then ethanol (two 5-ml portions); yield 70 mg (68%); mp 228–231° dec. Recrystallization of the product from water gave 31 mg of white crystalline solid with unchanged melting point; λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 280 mμ (16.9); pH 5.8, 277 mμ (18.0); pH 13, 281 mμ (20.3); ir (Nujol) $\bar{\nu}_{max}$ 3650 (NH), 2800–2300 (acidic H), 1750 (C=O), 1610, and 1500 cm^{-1} (C=C, C=N). Paper chromatography ($R_f \times 100$) showed A, 36; B, 37; C, 4.2; D, 45; E, 2.7.

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.4; H, 4.38; N, 33.8; ^{15}N, 3.48. Found: C, 46.2; H, 4.61; N, 33.6; ^{15}N, 3.5.

The melting point of this product was not depressed on admixture with the synthetic sample prepared from 6-chloropurine. The ir and uv spectra and the chromatographic mobility of the product are identical with those of the synthetic sample.

B. From 6-Chloropurine.—To a solution of 2.99 g (32.0 mmol) of sarcosine hydrochloride in 24.7 ml of 1 *N* sodium hydroxide were added 3.39 g (32.0 mmol) of anhydrous sodium carbonate and 2.47 g (16.0 mmol) of 6-chloropurine. The reaction mixture was refluxed for 3 hr and was then filtered in order to remove a small amount of residue. The filtrate was diluted with 40 ml of water, and the pH was adjusted to 3.0 with 88% formic acid. A white solid precipitated, was collected on a filter, and was washed with water (three 10-ml portions) and then ethanol (five 10-ml portions); yield 3.04 g (92%); mp 228–231° dec. Recrystallization of 100 mg of the material from water gave 62 mg of white needles with unchanged melting point; uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 280 mμ (17.0); pH 5.8, 277 mμ (17.3); pH 13, 282 mμ (22.1); ir (Nujol) $\bar{\nu}_{max}$ 3650 (NH), 2800–2300 (acidic H), 1750 (C=O), 1610, and 1550 cm^{-1} (C=C, C=N). Paper chromatography ($R_f \times 100$) showed A, 36; B, 57; C, 4.2; D, 48; E, 2.8; F, 48.

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.4; H, 4.38; N, 33.8. Found: C, 46.6; H, 4.67; N, 34.0.

(15) Initially, the ^{14}N compound was prepared by the same method, and was characterized by elemental analysis and ir and uv spectra.

(16) When the same reaction was carried out in buffer at pH 7, the products of the reaction were the same, but more adenine was formed.

Registry No.—**1**, 21333-80-2; **2**, 21371-69-7; **3**, 21343-09-9; **14**, 21343-10-2; **15**, 21343-11-3; **16**, 21371-70-0; *N*-benzyloxycarbonylsarcosine *p*-nitrophenyl ester, 16879-76-8.

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The Synthesis of 17-Deoxy-17-α and -17β 20-pregnynes and -20-pregnenes

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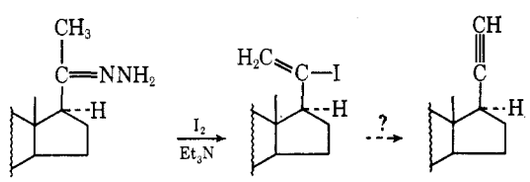
A variety of 17-deoxy-17α- and -17β-20-pregnynes, pregnenes, and pregnanes were prepared from 20-iodo-Δ^{20} steroids by base-catalyzed dehydrohalogenation. The mechanism of the elimination reaction from 20-iodo-Δ^{20} steroids to pregn-20-ynes is discussed.

Many pharmacologically active steroids contain the 17α-ethynyl-17β-hydroxy moiety (usually introduced by addition of a metal acetylide to a 17-keto steroid), and partial or complete reduction of the ethynyl group also produces compounds with biological activity.[1] Removal of the oxygen function at C-3 in many steroids enhances the original activity,[2] and it was of interest to see the effect of a similar loss in 17α-substituted 17β-hydroxy steroids. We, therefore, sought to prepare a series of 17-ethynyl-17-deoxy steroids and some simple reduction products.

Our synthetic approach made use of a reaction discovered by Barton some years ago,[3] but largely neglected since then. The reaction involves the treatment of a ketone hydrazone (*e.g.*, that of a 20-keto steroid) with iodine in the presence of triethylamine to afford high yields of vinyl iodide (*e.g.*, a Δ^{20}-20-iodo steroid). We felt that such a vinyl iodide should be

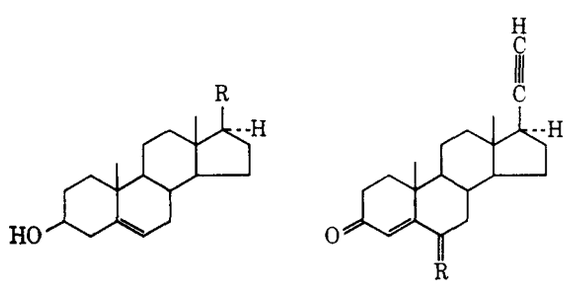


easily convertible, upon treatment with base, to the corresponding ethynyl compound. To test this idea, we decided to prepare some 17β-ethynyl steroids from readily obtainable 17β-acetyl (20-keto) steroids.

The starting material for this preliminary work was pregnenolone, which was converted to its hydrazone (hydrazine, triethylamine) and thence to the vinyl iodide (I) with iodine–triethylamine in tetrahydrofuran. This material was converted, upon refluxing with potassium hydroxide in methanol, to the acetylene II in 88% yield. The structure determination was made on the basis of infrared and nuclear magnetic resonance spectral data. The orientation of the ethynyl side chain was shown to be β (see below). The iodide I was also converted to the 17β-vinyl compound III by sodium–alcohol reduction.

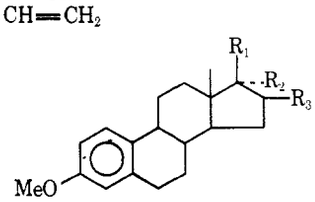
The desired conjugated ketone IV was prepared from II in 60% yield by Oppenauer oxidation. Jones oxidation afforded the undesired enedione V.

Since the above synthetic route to 17-ethynyl-17-deoxy steroids proved successful, we decided to prepare a few compounds in the A-ring aromatic series. The 20 ketone in this series was prepared *via* the 17-ethylidene compound,[4] by photosensitized oxygenation and dehydration,[5] and hydrogenation. This material was

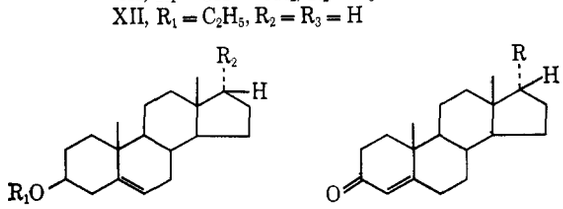


I, R = C(I)=CH$_2$
II, R = C≡CH
III, R = CH=CH$_2$

IV, R = H,H
V, R = O



VI, R_1, R_2 = O, R_3 = H
VII, R_1, R_2 = CHCH$_3$, R_3 = H
VIII, R_1 = COCH$_3$, R_2, R_3 = double bond
IX, R_1 = COCH$_3$, R_2 = R_3 = H
X, R_1 = C≡CH, R_2 = R_3 = H
XI, R_1 = CH=CH$_2$, R_2 = R_3 = H
XII, R_1 = C_2H_5, R_2 = R_3 = H



XIII, R_1 = R_2 = COCH$_3$
XIV, R_1 = H, R_2 = C(I)=CH$_2$
XV, R_1 = H, R_2 = C≡CH
XVI, R_1 = H, R_2 = CH=CH$_2$

XVII, R = C_2H_5
XVIII, R = C≡CH
XIX, R = CH=CH$_2$
XX, R = C_2H_5

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