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THE SYNTHESES AND MASS SPECTRA OF SOME *N*-SUBSTITUTED FERROCENYLMETHYL ADENINES

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Summary

Reaction of 6-chloropurine and ferrocenylmethylamine in methoxyethanol gives N^6-ferrocenyl adenine with concomitant formation of N^6,9-bis(ferrocenylmethyl)adenine. The formation of the latter compound can be suppressed by the addition of triethylamine in the reaction medium. Reaction of adenine with (ferrocenylmethyl)trimethylammonium iodide in boiling water gives 9- and 7-ferrocenylmethyladenine, as well as the N^6,9-dialkylated product. Details of the mass spectra of monoalkylated isomers have been recorded.

A variety of naturally occurring and synthetic N^6-substituted adenines exhibit physiological activities of cytokinins, a group of plant hormones which regulate cell division. N^6-Benzyladenine (BA), which is a commercially available synthetic cytokinin, is probably the best known cytokinin among many purely synthetic analogues. This is clearly due to the fact that BA can be relatively easily synthesized and that its activities compare favorably with a natural cytokinin. Thus several systematic studies on structure-reactivity relationship of BA homologues involving modification of the benzyl side chain have been reported [1,2]. In light of developing interest in biologically active organometallic compounds and certain similarities between a ferrocenyl group and a phenyl group in chemical reactivities and lipophilic properties, we have become interested in the synthesis of the ferrocene analogue of BA.

A prior, N^6-ferrocenylmethyladenine may be prepared by an alkylation of adenine with an appropriate ferrocenylmethyl derivative. It may be also synthesized by a nucleophilic displacement of 6-chloropurine by ferrocenylmethylamine. In the first method, masking of the most nucleophilic 9-amino-nitrogen in the adenine molecule with a suitable protective group is apparently necessary. It would appear, therefore, that the second synthetic method, which involves the reaction of 6-chloropurine and ferrocenylmethylamine, is relatively simple compared to an alkylation of adenine. Furthermore, the pattern of nu-

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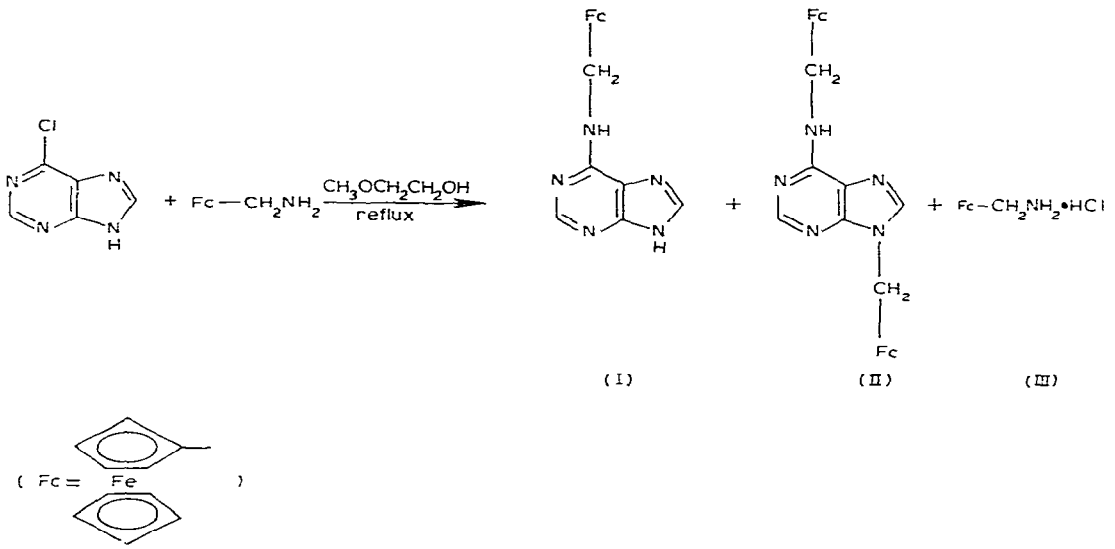
cleophilic displacement of 6-chloropurine is well known [3] and the starting materials can be either obtained commercially or easily prepared according to a known procedure. However, when 6-chloropurine and ferrocenylmethylamine were allowed to react in the presence of methoxyethanol at reflux temperature, the reaction led to a complex product mixture, and definable products which consisted of the expected N^6-ferrocenylmethyladenine (I) (21% yield), small amounts of N^6,9-bis(ferrocenylmethyl)adenine (II) and ferrocenylmethylamine hydrochloride (III). The unexpected occurrence of the dialkylated product II can be explained mechanistically: the II is a secondary product resulting from a nucleophilic attack of the primary product I on the amine hydrochloride III. It is well known that ferrocenylmethylammonium salt is a reactive electrophilic reagent [4].



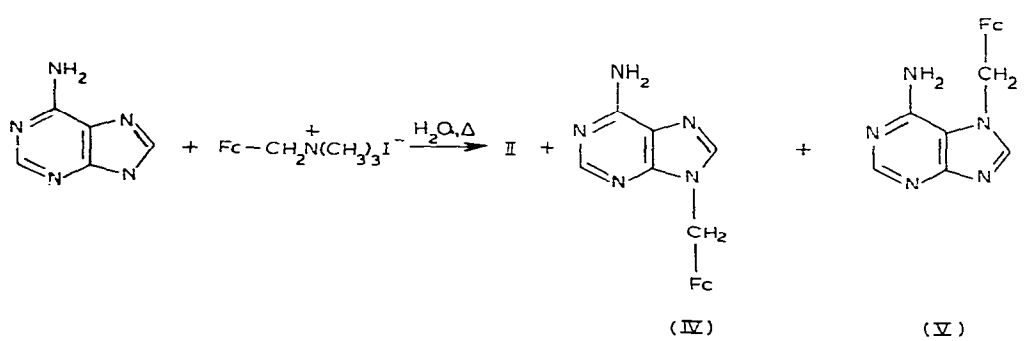
Both structures of I and II were established primarily using NMR and mass spectrometry. In the NMR spectrum of I (100 Hz, in DMSO-d_6) a broad two-proton singlet at δ 4.37 is assigned to the methylene group adjacent to the ferrocenyl substituent and is coupled to a D_2O exchangeable N^6-proton at δ 7.6. Two one-proton singlets at δ 8.09 and 8.22 may be assigned to the C(2) and C(8) protons, respectively [12]. A broad one-proton singlet at δ 12.85 is assigned to the C(9) amino-proton. The appearance of the proton signal at an unusually low-field region is clearly due to a strong H-bonding of the amino-proton to the sulfoxide group of the solvent. Observed resonance peaks due to the ferrocenyl substituent are in good agreement with well known data. In addition to the NMR and mass spectrometry analyses (see experimental section), the identification of the structure of compound II was further supported by a different synthetic route.

The above structural assignments are further verified by the UV spectrometric analysis. Compound I shows UV maxima (in 95% ethanol) at λ 206 nm (ϵ 30 × 10^3) and λ 267 nm (ϵ 22 × 10^3). The long-wave absorption exhibits bathochromic shift in both methanolic 0.1 *N* HCl (λ_{max} 275 nm) and 0.1 *N* NaOH (λ_{max} 272 nm) solutions. These UV characteristics are in accord with

those of a range of 6-substituted purines [14]. The short-wave absorption, however, shows a hypsochromic shift in acidic solution, but a bathochromic shift in base. Compound II has absorptions at λ_{max} 207 nm (ϵ 42 × 10^3) and λ_{max} 258 nm (ϵ 37 × 10^3). The long-wave absorption is relatively insensitive to pH, but the short-wave absorption has a slightly bathochromic shift in both basic and acidic solutions. The observed UV characteristics are in full agreement with that reported for 9-benzyl-6-benzylaminopurine [15].

In order to circumvent the problem of the formation of II at the expense of the desired monoalkylated product, an attempt was made to protect the 9-position with a 2-tetrahydropyranyl group [5]. This approach, however, did not significantly alter the product distribution since the protective group was easily cleaved by the hydrogen chloride generated in the reaction medium. Fortunately, when triethylamine was added as an acid scavenger, the formation of the reactive species III was greatly suppressed and the yield of I increased markedly to 48%. Although this method provides a synthesis of N^6-ferrocenylmethyladenine in a moderate yield, the preparation of a synthetic precursor, ferrocenylmethyl amine, using the known procedure [6] of reducing ferrocenylamide with lithium aluminium hydride, often gave an unsatisfactory yield. Consequently, the alternative method of synthesizing I by a direct alkylation of adenine was investigated.

In the first attempt, ferrocenylmethyl chloride was used as the alkylating agent. However, reaction of freshly prepared ferrocenylmethyl chloride [7] with 9-(tetrahydro-2-pyranyl)adenine gave an intractable mixture. This undesirable result is probably caused by the instability of ferrocenylmethyl chloride which decomposes readily at room temperature. This trait renders the compound not only difficult to prepare but also unsuitable as an alkylating agent. Meanwhile, in an exploratory study, directly alkylating adenine with (ferrocenylmethyl)trimethylammonium iodide in H_2O solution was investigated. This reaction proceeded smoothly to furnish three crystalline products. The main product was shown by NMR analysis to be 9-ferrocenylmethyladenine (IV), and a minor product was found to be identical to the compound II in every respect. Compound IV shows two UV maxima at λ 204 nm (ϵ 28 × 10^3) and λ 268 nm (ϵ 15 × 10^3), both of which are relatively insensitive to pH. The observed UV features of IV support that the adenine is alkylated at the 9-position of ring nitrogens [16]. The third product which was isolated in a trace amount is identified as 7-ferrocenylmethyl adenine (V). The structure of compound V is mainly deduced from the mass spectral fragmentation pattern of it, which together with spectra of compounds I and IV are reproduced in Fig. 1.



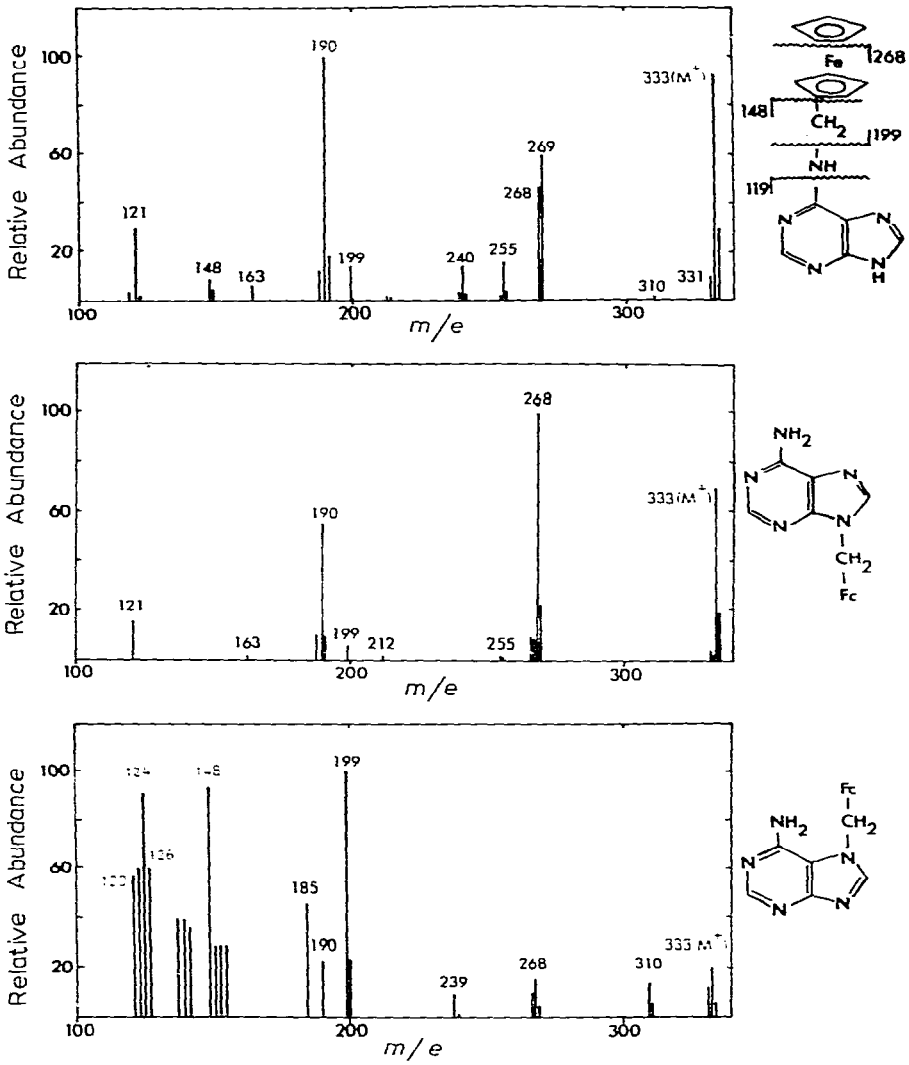
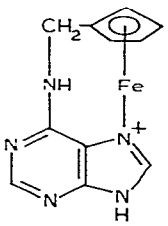


Fig. 1. Mass spectra (70 eV) of 3 isomeric *N*-substituted ferrocenylmethyladenines.

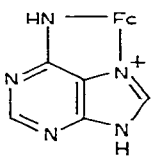
The fragmentations of all three *N*-substituted ferrocenylmethyladenine isomers can best be illustrated by those of compound I. Similar to the known pattern of various 6-substituted aminopurines [8], fragmentation of the ferrocenylmethyl side chain prevails in structure I. In the upper mass range, loss of the cyclopentadienyl radical from the molecular ion to give ion *m/e* 268 is observed which is supported by the presence of a metastable ion at 215.7. The species *m/e* 268 may be depicted as VI which may subsequently fragment to VII by ejection of a fulvene or a benzene ring to give the most intense peak at *m/e* 190. A similarly stabilized bridged ion has been postulated in the fragmentation of a naturally occurring cytokinin, zeatin [9]. The mass spectrum of IV is generally similar to that of I, but exhibits the most abundant ion peak at

m/e 268 which may be represented by ion VIII. In contrast to compounds I and IV, V shows major peaks at a lower mass range; the main degradation processes are represented by the formation of the well-known ferrocenylmethyl cation (*m/e* 199) [13] and loss of the ferrocenyl group to give ion IX (*m/e* 148). Such a preferential elimination pattern in V is clearly due to the steric repulsion resulting from two *peri* substituents on the purine molecule. Furthermore, it may be envisaged that steric interaction between the 7-ferrocenylmethyl substituent and the 6-amino group would facilitate formation of a highly stabilized ferrocenylmethyl cation (*m/e* 199).



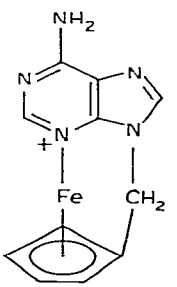
m/e 268

(VI)



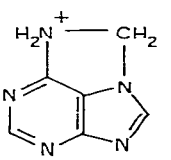
m/e 190

(VII)



m/e 268

(VIII)



m/e 148

(IX)

The aforementioned product analysis of the reaction of adenine with (ferrocenylmethyl)trimethylammonium iodide agrees with the view that alkylation under alkaline conditions in aqueous media gave either the 9-alkyladenine or a mixture of 7- and 9-alkyl isomers [3,10]. It must be pointed out that, in certain cases, the minor product obtained under similar conditions was found to be the 3-alkylated adenine [11]. Although the structure assignment of the minor monoalkylated product is not rigorously proven in the present study, the possibility of the N^7-isomer prevails, as is evidenced from the mass spectral analysis. It must be mentioned that, at 70 eV, considerable thermal degradations of the compounds were occurring during the spectrometric analysis since both mass spectra of compounds I and V showed at least one peak, *m/e* 310, revealing the samples to be thermally decomposed. This common culprit in artifact production is clearly due to the polarity of compounds and the effect of hot filament on the temperature of the ion source. This observation was supported by the measurement of a spectrum at 15 eV immediately after that of 70 eV spectrum. There were absences of the *m/e* 310 peaks in both spectra of the compounds I and V, as well as numerous fragment ions of low mass in V (see Experimental section).

The foregoing results suggest that (ferrocenylmethyl)trimethylammonium iodide can be used favorably as an alkylating agent for the preparation of compound I, provided the 9-position of adenine molecule is blocked. However, at this stage of study, a biological assay of I shows no cytokinin activity. On the contrary, I exhibited a suppressive effect on cell division at a very low concentration (10^{-6} *M*). Thus no further studies to find an effective procedure to synthesize N^6-ferrocenylmethyladenine were performed.

Experimental

Reaction of ferrocenylmethylamine with 6-chloropurine

A 5-ml flask fitted with a reflux condenser and a nitrogen inlet was charged with ferrocenylmethylamine (278 mg; 1.3 mmol), 6-chloropurine (200 mg, 1.3 mmol), and methoxyethanol (2 ml), and the whole, purging with the nitrogen gas, was heated to reflux temperature on an oil bath. After heating for 2 h, the oil bath and the condenser were removed and rapidly flushed with the nitrogen gas to evaporate most of the solvent. The residue was then treated with methanol (10 ml) to give a yellow precipitate (86 mg), which was recrystallized from acetonitrile/methanol mixture to give N^6-9-ferrocenylmethyl adenine (II), m.p. 243°C (dec.). FTPMR (100 Hz, in $CDCl_3$) δ 4.20 (s, 5H, Cp), 4.22 (s, 5H, Cp), 4.2—4.3 (m, 8H, substituted Cp rings), 4.52 (d, J 3 Hz, 2H, HN—CH_2), 5.90 (broad, 1H, NH), 7.67 (s, 1H, =N—CH=), and 8.47 (s, 1H, =N—CH=). MS (15 eV): 531 (100%), 332 (26%), 199 (28%).

After isolation of compound II, the remaining mother-liquor was treated with petroleum ether to precipitate N^6-ferrocenylmethyladenine (I) (90 mg), which was then recrystallized twice from methanol/petroleum ether to give crystals of I; m.p. 240—245°C (dec.). MS (15 eV) 333 (100%), 268 (5%), 135 (4%). Elemental analysis Found: C, 57.62; H, 4.54; N, 21.22. $C_{16}H_{15}N_5Fe$ calcd.: C, 57.70; H, 4.50; N, 21.02%. The final mother-liquor was set aside overnight at room temperature to give fine yellow crystals (72 mg), which were recrystallized from methanol/chloroform to give compound III, m.p. 174—176°C (dec.). The IR spectrum of III was superimposable on that of an authentic sample of ferrocenylmethylamine hydrochloride.

In a separate experiment which was carried out under conditions similar to those described above except 0.3 ml of triethylamine was added to the starting materials, the concentrate product mixture gave 203 mg of crude compound I.

Reaction of adenine with (ferrocenylmethyl)trimethylammonium iodide

A solution of adenine (270 mg, 2 mmol) and (ferrocenylmethyl)trimethylammonium iodide (770 mg, 2 mmol) in 100 ml of distilled water was heated to reflux temperature under nitrogen atmosphere. Evolution of trimethylamine began after the onset of boiling. The heating was continued for 6 h and then the solution was allowed to cool to room temperature. The yellow solid that separated from the reaction mixture was removed by filtration. An alumina TLC analysis of the solid substance showed that it was a mixture of three products. Column chromatographic separation of the mixture over the neutral alumina using chloroform as eluent gave pure N^6,9-bis(ferrocenylmethyl)-adenine (110 mg) as the first eluate, followed by a band which gave a small number of yellow crystals of V, (ca. 3 mg), m.p. 202°C (dec.). MS (15 eV): 333 (100%), 268 (18%), 267 (18%), 214 (23%), 199 (68%), 148 (25%). The next eluent, 1% methanol in $CHCl_3$ gave pure 9-ferrocenylmethyladenine (250 mg), m.p. 242—244°C (dec.). NMR (60 MHz, in DMSO-d_6) δ ~4 (m, overlapped, 2H, α-protons of substituted Cp), 4.35 (t, J 2 Hz, 2H, β-protons of substituted Cp), 4.15 (s, 5H, Cp), 5.1 (s, 2H, NH_2), 7.15 (broad, 2H, D_2O exchangeable), and 8.13 (s, 2H, two N=CH—N=). MS (15 eV): 333 (100%), 268 (16%), 200 (43%), 199 (12%) and 186 (14%).

Mass spectra measurements

The mass spectra were taken with a Perkin—Elmer 270 GC/MS Spectrometer using the direct insertion probe at the ion source temperature ranging from 70 to 110°C. The 15 eV spectra were recorded after the measurements of the 70 eV spectra.

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References

1 S. Kuraishi, Sci. Pap. Coll. Gen. Educn., Univ. Tokyo, 9 (1959) 67.
2 F. Skoog, H.Q. Hamzi, A.M. Sweykowska, M.J. Leonard, K.L. Carraway, T. Fujii, J.P. Helgeson and R.N. Leoppky, Phytochemistry, 6 (1967) 1169.
3 L.H. Lister in D.J. Brown (Ed.), Fused Pyrimidines Part II: Purines, Wiley-Interscience, New York, pp. 152—160.
4 D. Lednicer, J.K. Lindsay and C.R. Hauser, J. Org. Chem., 23 (1958) 653.
5 R.K. Robins, E.F. Godefroi, E.C. Taylor, L.R. Lewis and A. Jackson, J. Amer. Chem. Soc., 83 (1961) 2574.
6 K. Schloegel, Monatsh. Chem., 88 (1957) 601.
7 T.T. Tidwell and T.G. Traylor, J. Amer. Chem. Soc., 88 (1966) 3442.
8 H. Budzikiewicz, C. Djerassi and D.H. Williams, Mass Spectrometry of Organic Compounds, Holden-Day, San Francisco, 1967, pp. 592—594.
9 D.S. Letham, J.S. Shannon and I.R.C. McDonald, Tetrahedron, 23 (1967) 479.
10 E.P. Lira and C.W. Huffman, J. Org. Chem., 31 (1966) 2188.
11 L.B. Townsend, R.K. Robins, R.N. Loeppky and N.J. Leonard, J. Amer. Chem. Soc., 96 (1964) 5320.
12 J.H. Keck, Jr., R.A. Simpson and J.L. Wong, J. Org. Chem., 43 (1978) 2587.
13 H. Egger, Monatsh Chem., 97 (1966) 602.
14 M.W. Bullock, L.J. Hand and E.L.R. Stockstad, J. Amer. Chem. Soc., 78 (1956) 3693.
15 N.J. Leonard, K.L. Carraway and J.P. Helgeson, 2 (1965) 291.
16 L.B. Townsend, R.K. Robins, R.N. Loeppky and N.J. Leonard, J. Amer. Chem. Soc., 86 (1964) 5320.