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STRUCTURE OF DISCADENINE, A SPORE GERMINATION INHIBITOR FROM THE CELLULAR SLIME MOLD, DICTYOSTELIUM DISCOIDEUM

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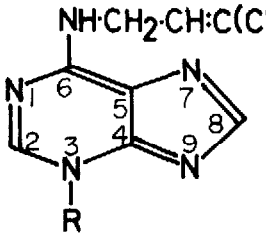
In the previous papers, we reported the isolation, purification and some nature of a potent spore germination inhibitor from the cellular slime mold, Dictyostelium discoideum,[1] and subsequently presented the partial structure consisting of 6-(3-methyl-2-butenylamino)purine moiety and an unknown α-amino acid residue which was most possibly substituted at the N-3 position of the purine ring.[2] Recently we have developed an improved isolation procedure described herein for structure determination. In this paper, we wish to present the structure of the inhibitor which we name discadenine, as 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenylamino)purine(I).

Discadenine was extracted from spores with 80% ethanol and subsequently was subjected to dialysis after removal of the alcohol under reduced pressure following the previous procedure.[2] The dialyzate was passed through an Amberlite XAD-2 column and eluted with aqueous ethanol. The activity appeared in fractions of 20-50% ethanol in water.

This procedure is proved to be a simple and effective purification method of discadenine. Those fractions were chromatographed on Sephadex LH-20 using 80% ethanol as



I: $CH_2CH_2CHCOOH$ (NH_2) (discadenine)

II: CH_3

the eluent. About 15 mg (colorless needles, m.p. 205-7°C) of discadenine was obtained from spores collected from twenty thousands of Petri plates. As a model compound of N_6, 3-disubstituted adenine moiety, 3-methyl-6-(3-methyl-2-butenylamino)purine (II, m.p. 175-6°C) was synthesized by reaction of 6-methylthio-3-methylpurine with 3-methyl-2-butenylamine.

The uv spectra of discadenine(I) resemble closely those of II (Table 1).

Table 1. Ultraviolet absorption spectra of discadenine(I) and 3-methyl-6-(3-methyl-2-butenylamino)purine(II).

	pH	max(nm)	$\varepsilon \times 10^{-3}$	min(nm)	$\varepsilon \times 10^{-3}$
I.....	2	287.0	22.5	241.5	3.3
	7	288.9	17.5	248.3	3.5
	12	290.0	17.4	248.7	3.5
	MeOH	292.7	15.7	248.9	3.1
II.....	2	285.2	22.4	240.0	2.8
	7	286.7	18.0	246.7	2.5
	12	287.7	17.6	247.8	2.8
	MeOH	291.7	16.4	247.8	2.4

Figure 1(B) shows the ^{13}C nmr spectrum[3] of discadenine (14 signals as numbered) taken at pD 3.4. The ten peaks (2-8, 11 and 13-14) were the same as corresponding peaks of II (Fig.1 C) within experimental conditions ($\pm$ 0.2 ppm), suggesting that these peaks arose from purine-ring and 3-methyl-2-butenyl carbons. Five extremely low-intense peaks, 1,2,4,6 and 8 (of Fig.1 B) were readily ascribed to a carbonyl carbon and quarternary carbons. The reasons for such low-intensities are partly due to lack of nuclear Overhauser enhancement by proton decoupling and partly due to shorter pulse-repetition times (2 sec) compared with their longer relaxation times ($\sim$15 sec).[4] Thus, the peak-assignments, given in Fig.1(B), are straightforward together with the additional information about the structure from ^{13}C-H spin-couplings (Fig.1 A). The relative positions of C_2 and C_8 are confirmed in the light of the differences in the C-H coupling constants.[5] The individual peak-assignments of C_4-C_6 (to peaks 2,4,6), however, were not attempted here, although the assignment might be possible by measurement of spin-lattice relaxation times.[4]

Subsequently, the four peaks marked by the arrows (Fig.1 B) should be

assigned to the carbons of the amino acid, the presence of which was confirmed by ninhydrin reactions.[2] The proton-coupled spectrum, peak 9 (doublet), peaks 10 and 12 (triplets) (of Fig.1 A),indicated that the possible structure of the amino acid moiety should be limited to $CH_2CH_2CH(NH_2)COOH$. Consequently, peaks 1,9,10 and 12 are assigned to the carbons of COOH, α-CH, γ-CH_2 and β-CH_2, respectively. The above results indicate that discadenine is 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenylamino)purine(I).

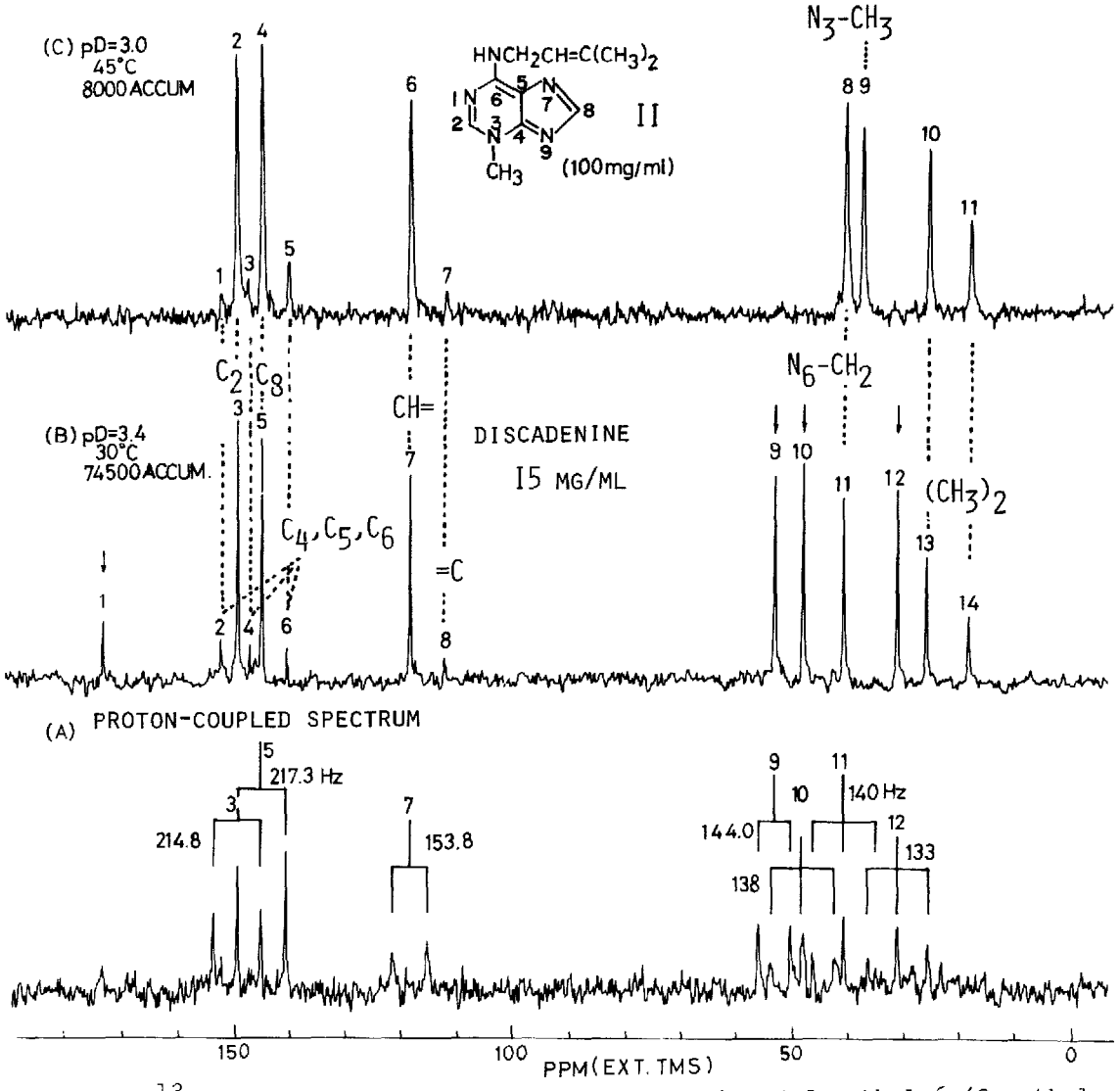
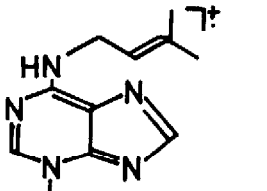
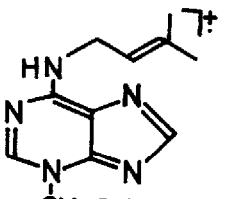
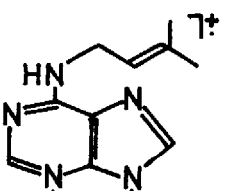


Figure 1. ^{13}C nmr spectra of discadenine (A and B) and 3-methyl-6-(3-methyl-2-butenylamino)purine (II)(C).

The structure of discadenine was supported by the high resolution mass measurement[6]: M^+, $C_{14}H_{20}N_6O_2$ (found 304.1657; calcd 304.1648), $C_{13}H_{20}N_6$ (found 260.1761: calcd 260.1749), $C_{12}H_{16}N_5$ (found 230.1429; calcd 230.1406) and $C_{11}H_{15}N_5$ (found 217.1348; calcd 217.1327). The structures of fragment ions are shown below. The fragment pattern below m/e 203 was similar to that of 6-(3-







methyl-2-butenylamino)purine.[7]

Discadenine is the first natural purine derivative possessing α-amino acid residue on the 3-position of the purine ring. Besides its pronounced spore germination inhibitory activity, discadenine exhibits significant cytokinin activity (approximately two-thirds level of kinetin at 2.3×10^{-7}M) in the standard tobacco pith test.

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REFFERENCES AND NOTES

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