IDENTIFICATION OF ADDUCTS FORMED BY REACTION OF PURINE BASES WITH A MUTAGENIC ANTHRAQUINONE, LUCIDIN: MECHANISM OF MUTAGENICITY BY ANTHRAQUINONES OCCURING IN RUBIACEAE PLANTS

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A mutagenic anthraquinone, lucidin (**1**), occurring in Rubiaceae plants, reacted with nucleic bases under physiological conditions to form adducts. The order of the reactivity is as follows: adenine > guanine >> pyrimidine bases ~ 0. By spectroscopic analyses, the isolated purine base adducts were identified as condensed reactants at the benzylic position of **1** with a nitrogen atom of a purine base. The results indicate a strong possibility of the formation of an exomethylenic compound as an electrophile intermediate.

KEYWORDS lucidin; *Rubia tinctorum*; adduct formation; mutagenicity; Rubiaceae

The Rubiaceae plants, which produced anthraquinones as secondary metabolites, have been used as a source of natural food colorant and traditional medicine in the Orient and the Occident.[1] In 1979, Brown and Dietrich[2] first found that lucidin (**1**), which is a major Rubiaceae anthraquinone, is mutagenic. Subsequently, several authors[3] showed the genotoxic effects of anthraquinones isolated from Rubiaceae plants. In the preceding paper[3f] we reported that the mutagenicity of the anthraquinones occurring in Rubiaceae plants strongly relates to their structures.[4] In order to elucidate the mechanism of the mutagenicity and explain the structure-mutagenicity relationships, we carried out a study of the reaction of **1** and nucleic bases since **1** is the most potent direct mutagen among Rubiaceae anthraquinones. In this paper, we deal with the reactions and structural determinations of the purine base adducts. In addition, we propose an exomethylenic intermediate which could determine the structure-mutagenicity relationship.

An aqueous solution (5 ml, pH 7) containing **1** (1 mg), polyvinylpyrrolidone (10 mg) and a nucleic base (each 5 mg) was incubated at 37°C for 20 days. Adduct formation was monitored by analytical HPLC.[5] The HPLC revealed that reaction of **1** with adenine or guanine furnishes two adducts each and that the reactivity of adenine is better than that of guanine. No pyrimidine base adduct was detected at any time during the course of the reaction. In order to prepare substantial amounts of the adducts for spectroscopic analyses, the reactions of **1** (5 mg) with a purine base (25 mg) in DMSO (5 ml) at 37°C were performed, and chromatographic purification of the resulting solutions afforded LA-1, LA-2 (each **1**-adenine adduct) and LG-2 (**1**-guanine adduct).

FAB-MS (pos./*m*-NBA) analyses of LA-1, LA-2, and LG-2 showed quasi-molecular ion peak at *m/z* 388 $[M+H]^+$, at *m/z* 388 $[M+H]^+$ and at *m/z* 404 $[M+H]^+$, respectively. The high-resolution MS analyses of the peaks gave the quasi-molecular formula as $C_{20}H_{14}O_4N_5$ (calcd. 388.1045) for LA-1

(observed 388.1032) and LA-2 (observed 388.1018) and as $C_{20}H_{14}O_5N_5$ (calcd. 404.0995) for LG-2 (observed 388.1018). These data suggested that the adducts were condensed compounds between one molecule of **1** and one molecule of each base with dehydration. Furthermore, ^{1}H-NMR[6] (500 MHz) and ^{13}C-NMR spectra[7] (125 MHz) measured in DMSO (LA-2 and LG-1) or DMSO-0.1N DCl (9:1) (LA-1) support the elucidation.

In order to determine where the lucidin moiety connects to the base moiety in each adduct, heteronuclear multiple quantam coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) analyses were performed. In the HMQC spectrum of LA-1 proton signals of adenine moiety at δ 8.73 and δ 8.47, both of which should be assigned as 2-position or 8-position, were correlated to carbon signals at δ 149.8 and δ 144.7, respectively. In the HMBC spectrum, cross-peaks were observed between proton signal at δ 8.73 and quaternary carbon signals at δ 153.8 and δ 147.5; and between a proton signal at δ 8.47 and quaternary carbon signals at δ 147.5 and δ 110.2. Thus, the carbon signals of adenine moiety were fully assigned as described in the references and notes. In the HMBC spectrum a cross-peak between the benzylic proton signal at δ 5.62 and carbon signals at δ 149.8 and δ 147.5 were also observed. Therefore, the benzylic carbon in lucidin moiety should be connected to the nitrogen at 3-position in adenine moiety, and the structure of LA-1 is determined as 3-[(1,3-dihydroxyanthraquinon-2-yl)methyl]adenine (Fig. 1). In the cases of LA-2 and LG-1, signal assignments were also performed in the same way as described for LA-1, and their benzylic proton signals were correlated to the carbon signals of 5-position and 8-position in adenine moiety and the carbon signals of 4-position and 8-position of guanine moiety, respectively. Therefore the structures of LA-2 and LG-1 are determined as 7-[(1,3-dihydroxyanthraquinon-2-yl)methyl]adenine and 9-[(1,3-dihydroxyanthraquinon-2-yl)methyl]guanine (Fig. 1), respectively.

It is believed that an electrophile is the key compound in the elucidation of the mechanism of mutagenicity, since nucleophilic attack by a nucleic base in DNA to an electrophile triggers the mutation in most cases. The present finding showed that the adducts are formed with dehydration on reaction of the benzylic carbon of **1** with a nitrogen atom of purine bases. It has been reported[3f,8] that **1** reacts with methanol or ethanol to form methyl or ethyl ether at its benzylic position. In EI-MS of **1** the dehydrated fragment ion at *m/z* 252 [M-18] was observed as base peak. These results suggest that the exomethylenic compound as an electrophile easily arises from **1** with dehydration. The proposed pathway for the formation of the adducts via the exomethylenic electrophile is shown as Fig. 2.

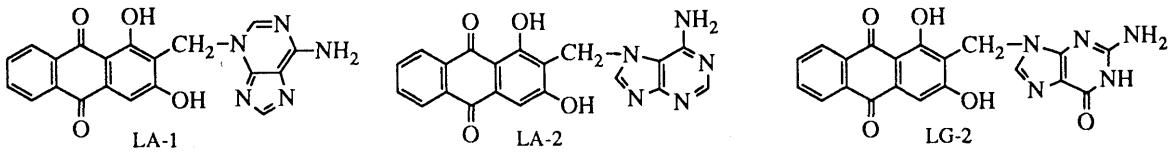

Fig. 1. Structures of Lucidin-Base Adducts

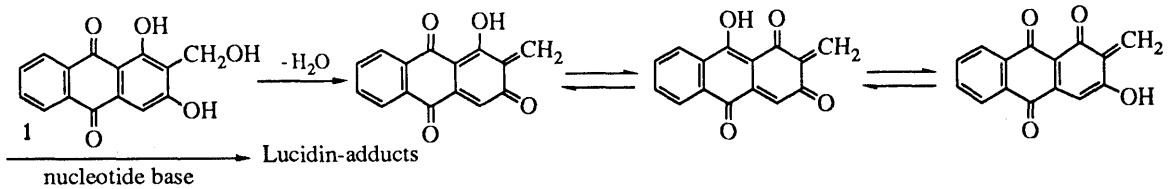

Fig. 2. Proposed Pathway for the Formation of Lucidin-Adducts

The molecular requirement for the formation of the electrophile and the ease of the formation well illustrate the structure-mutagenicity relationships. A compound such as nordamnacanthal (2-formyl-1,3-dihydroxyanthraquinone), munjistin (2-carboxy-1,3-dihydroxyanthraquinone), and 2-hydroxymethyl-anthraquinone, which does not possess an oxymethylene group on carbon 2 or a hydroxy group on carbon 1 or 3, are non-mutagenic because of the difficulty of the formation of the exomethylenic electrophile. **1** shows strong mutagenicity since the tautomerization as shown in Fig. 2 accelerates the dehydration to the exomethylenic electrophile. The substituion of the 1,3-dihydroxy group drastically decreases the mutagenicity because the exomethylenic electrophile derived from the substituted compounds such as damnacanthol (lucidin-1-*O*-methyl ether) and lucidin-3-*O*-primeveroside cannot exist as the stable tautomer.

1 is a planar aromatic compound; therefore, the lucidin residue of the adducts seems to easily enter the groove in DNA, leading to perturbation of the DNA structure. Further studies are needed to clarify the more detailed mechanism of the mutagenicity by lucidin derivatives.

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4) The structure-mutagenicity relationships are as follows. 1,3-Dihydroxyanthraquinones possessing an oxymethylene group on carbon 2 show strong direct mutagenicity. The oxygenated state of the benzylic carbons and the number of hydroxyl groups on carbon 1 and carbon 3 are important in determination of the mutagenicity.
5) "HPLC condition" column: Capcell pak C18 (250 x 4.6 mm i.d.); flow rate: 1 ml/min; temperature: 40°C; detection: 260 nm; solvents: MeOH-H_2O-AcOH (6/4/0.3).
6) LA-1, DMSO-d_6 / 0.5 N DCl (9/1), δ : 5.62 (2H, s, CH_2), 7.23 (1H, s, L-H4), 7.87 (2H, m, L-H6 and L-H7), 8.08 (1H, d, J = 7 Hz, L-H8), 8.14 (1H, d, J = 7 Hz, L-H5), 8.47(1H, s, A-H8), 8.73(1H, s, A-H2); LA-2, DMSO-d_6, δ : 5.50 (2H, s, CH_2), 7.19 (1H, s, L-H4), 7.20 (1H, s, NH_2), 7.86 (2H, m, L-H6 and L-H7), 8.12 (1H, m, L-H8), 8.13 (1H, s, A-H2), 8.14 (1H, s, A-H8), 8.17 (1H, m, L-H5), 13.6 (1H, s, L-C1-OH); LG-2, DMSO-d_6, δ : 6.44 (2H, s, CH_2), 7.27 (1H, s, L-H4), 7.42 (1H, s, G-H8), 7.92 (2H, m, L-H5 and L-H7), 8.17 (1H, m, L-H8), 8.23 (1H, m, L-H5), 10.50 (1H, s, G-NH), 13.4 (1H, s, L-C1-OH).
7) LA-1, DMSO-d_6 / 0.5 N DCl (9/1), δ: 43.2 (CH_2), 107.8 (L-4), 109.8 (L-9a), 110.2 (A-5), 112.8(L-2), 127.0 (L-5 or L-8), 127.2 (L-5 or L-8), 133.0 (L-10a or L-8a), 133.5 (L-10a or L-8a), 135.2 (L-4a), 135.6 (L-6 and L-7), 144.7 (A-8), 147.5 (A-4), 149.8 (A-2), 153.8 (A-6), 163.3 (L-1 or L-3), 164.2 (L-1 or L-3), 182.0 (L-10), 186.8 (L-9); LA-2, DMSO-d_6, δ: 40.0 (CH_2), 107.5 (L-4), 108.9 (L-9a), 111.1 (A-5), 113.9(L-2), 126.3 (L-5 or L-8), 126.7 (L-5 or L-8), 132.8 (L-4a, and L-10a or L-8a), 133.1 (L-10a or L-8a), 134.3 (L-6 or L-7), 134.6 (L6 or L-7), 146.1 (A-8), 151.6 (A-6), 151.7 (A-2), 158.3 (A-4), 163.2 (L-1 or L-3), 165.7 (L-1 or L-3), 181.9 (L-10), 185.5 (L-9); LG-2, DMSO-d_6, δ: 34.6 (CH_2), 107.8 (L-4), 109.2 (L-9a), 114.3 (L-2), 116.2(G-5), 126.5 (L-5 or L-8), 126.8 (L-5 or L-8), 132.8 (L-10a or L-8a), 133.0 (L-10a or L-8a), 133.8 (L-6 and L-7), 134.3 (L-4a), 136.5 (G-8), 151.1 (G-4), 153.4 (G-2), 156.6 (G-6), 163.1 (L-1 or L-3), 163.9 (L-1 or L-3), 181.8 (L-10), 186.2 (L-9).
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