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# Alkylation of Nucleosides by Dehydromonocrotaline, the Putative Toxic Metabolite of the Carcinogenic Pyrrolizidine Alkaloid Monocrotaline

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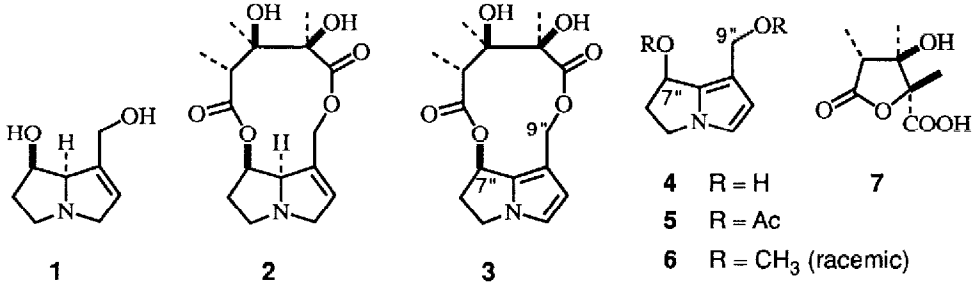
**Abstract.** Reaction of dehydromonocrotaline (**3**), the putative toxic metabolite of the carcinogenic pyrrolizidine alkaloid monocrotaline (**2**), with various nucleosides proceeded mostly at the C-9" position of **3** to give several nitrogen atom-alkylated nucleosides including N-7 alkylated 2'-deoxyguanosine **14**.

Pyrrolizidine alkaloids having retronecine (**1**) or otonecine as the necine base portion exhibit marked hepatotoxicity and, in certain cases, carcinogenicity and antitumor activity.[1] In particular, monocrotaline (**2**) found in various species of *Crotalaria* plants is the best-known carcinogenic pyrrolizidine alkaloid and sometimes contaminates certain medicinal herbs and foodstuffs such as milk and honey.[1] The highly reactive pyrrolic metabolite, dehydromonocrotaline (**3**) acting as a bifunctional electrophile is supposed to be responsible for the acute (liver lesion) and the chronic (carcinogenicity) toxicity.[1] DNA cross-linking *in vivo* and *in vitro* has been observed with monocrotaline (**2**), dehydromonocrotaline (**3**), dehydroretronecine (**4**), and the diacetate **5**.[2] Dehydroretronecine (**4**) is known to alkylate several nucleosides and nucleotides.[3] However, only limited chemical studies have been reported on the putative toxic metabolite dehydromonocrotaline (**3**).[4] Herein we describe the first evidence that highly reactive dehydromonocrotaline (**3**) directly alkylates various nucleosides to give several adducts including N-7 alkylation product of 2'-deoxyguanosine **14**.

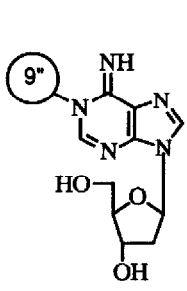
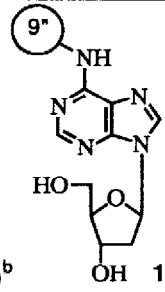
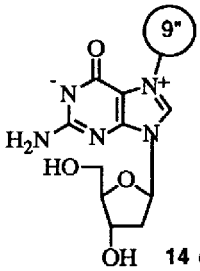
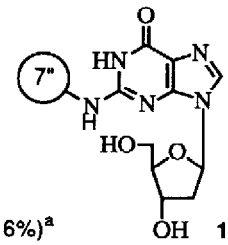
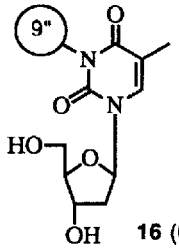
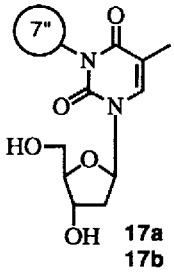
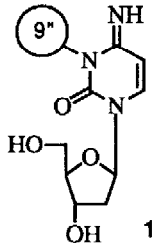


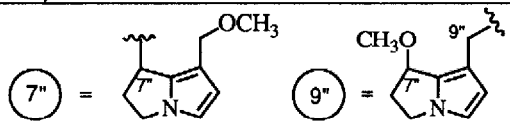

**1** **2** **3**

**4** R = H **7**
**5** R = Ac
**6** R = $CH_3$ (racemic)

Dehydromonocrotaline (**3**) was prepared from monocrotaline (**2**) by the reported procedure.[5] Prior to reaction of **3** with nucleosides, we investigated the stability of **3** in protic solvents. Thus, dehydromonocrotaline (**3**) was found to be hydrolyzed within 1 min in the aqueous media at room temperature. However, it survived for more than 5 h in the $CH_3OH$ solution in the presence of $NaHCO_3$ as an acid scavenger. The reaction of dehydromonocrotaline (**3**) with 1 equiv of each nucleoside (**8-11**) was therefore performed in $CH_3OH$ (20 mM) containing 4 equiv of $NaHCO_3$ at 25 °C for 5 h. After filtration and concentration of the reaction mixture, the

**Table I. Alkylation of Nucleosides with Dehydromonocrotaline (3)**

| nucleoside | alkylated nucleoside (yield) |
|---|---|
| 2'-deoxyadenosine (**8**) |  **12** (0.7%)[b]  **13** (0 2%)[a] |
| 2'-deoxyguanosine (**9**) |  **14** (0 6%)[a]  **15a** (0.2%) **15b** (0.2%) |
| thymidine (**10**) |  **16** (0.02%)[a]  **17a** (0 1%) **17b** (0 1%) |
| 2'-deoxycytidine (**11**) |  **18** (1.0%)[b] |



[a] This material consists of a single diastereomer. Stereochemistry of the methoxyl group at the C-7" was not determined. [b] A 1:1 mixture of diastereomers concerning the C-7" methoxyl group

**Table II. UV Spectra of Alkylated Nucleosides 12-18 and Methylated and Ethylated Nucleosides**

| nucleoside | alkylated nucleoside | pH 1[a] λmax (nm) | pH 1[a] λmin (nm) | pH 7[b] λmax (nm) | pH 7[b] λmin (nm) | pH 13[c] λmax (nm) | pH 13[c] λmin (nm) |
|---|---|---|---|---|---|---|---|
| 2'-deoxy-adenosine (**8**) | **12** | 258 | 237 | 258 | 238 | 260 (268 sh) | 238 |
| | 1-Et-adenosine[d] | 259 | 235 | 259 | 235 | 261 (268 sh) | 237 |
| | **13** | 265 | 238 | 268 | 240 | 268 | 240 |
| | $N^6$-Et-adenosine[d] | 264 | 239 | 268 | 242 | 268 | 243 |
| 2'-deoxy-guanosine (**9**) | **14** | 256 (278 sh) | 237 | 256 (278 sh) | 243 | | |
| | 7-Et-guanosine[e] | 257 (277 sh) | 244 | 257 (277 sh) | 235 | | |
| | **15a** | 258 (282 sh) | 235 | 255 (277 sh) | 230 | 259 (269 sh) | 242 |
| | **15b** | 258 (282 sh) | 235 | 255 (277 sh) | 231 | 260 (269 sh) | 243 |
| | $N^2$-Me-guanosine[f] | 258 (283 sh) | 233 | | | 258 (270 sh) | 237 |
| thymidine (**10**) | **16** | 267 | 246 | 267 | 245 | 267 | 245 |
| | **17a** | 268 | 246 | 268 | 244 | 269 | 245 |
| | **17b** | 268 | 246 | 268 | 244 | 269 | 245 |
| | 3-Me-thymidine[g] | 265 | 236 | 266 | 237 | 267 | 238 |
| 2'-deoxy-cytidine (**11**) | **18** | 280 | 246 | 280 | 246 | 267 | 247 |
| | 3-Et-cytidine[h] | 280 | 247 | 279 | 246 | 267 | 248 |

[a] $HCl/H_2O$-MeOH (8:2). [b] $NH_4OAc/H_2O$-MeOH (8:2). [c] $NaOH/H_2O$-MeOH (8:2). [d] Singer, B.; Sun, L.; Fraenkel-Conrat, H. *Biochemistry* **1974**, *13*, 1913. [e] Singer, B *Biochemistry* **1972**, *11*, 3939. [f] Smith, J. D.; Dunn, D. B. *Biochem. J.* **1959**, *72*, 294. [g] Friedman, O. M.; Mahapatra, G. N.; Dash, B.; Stevenson, R. *Biochim. Biophys Acta* **1965**, *103*, 286. [h] Sun, L.; Singer, B. *Biochemistry* **1974**, *13*, 1905.

**Table III. Selected $^1H$ NMR Spectral Data for Alkylated Nucleosides 12-18 and dehydroretronecine dimethyl ether (6)[a]**

| compound | chemical shifts of H-7" and H-9" protons H-7" | H-9" | | | reacting site of **3** |
|---|---|---|---|---|---|
| **6** | 4.77 (1 H, dd, 6.3, 1.7) | 4.33 (1 H, d, 11.5) | 4.41 (1 H, d, 11.5) | | - |
| **12** | 4.79 (0.5 H, dd, 3.6, 2.0)† | 5.07 (0.5 H, d, 14.8) | 5.17 (0.5 H, d, 14.8) | | C-9" |
| | 4.81 (0.5 H, dd, 3.6, 2.0)† | 5.08 (0.5 H, d, 14.8) | 5.18 (0.5 H, d, 14.8) | | C-9" |
| **13** | 4.82 (1 H, dd, 6.0, 1.5) | -* | 4.99 (1 H, d, 15.9) | | C-9" |
| **14** | -* | 5.57 (1 H, d, 12.0) | 5.64 (1 H, d, 12.0) | | C-9" |
| **15a** | 5.52 (1 H, dd, 7.6, 4.3) | 4.27 (1 H, d, 10.8) | 4.32 (1 H, d, 10.8) | | C-7" |
| **15b** | 5.53 (1 H, dd, 7.6, 4.3) | 4.27 (1 H, d, 11.2) | 4.31 (1 H, d, 11.2) | | C-7" |
| **16** | -* | -* | 5 08 (1 H, d, 14.0) | | C-9" |
| **17a** | 6.50 (1 H, br d, 9.0) | 4.03 (1 H, d, 11.0) | 4.20 (1 H, d, 11.0) | | C-7" |
| **17b** | 6.50 (1 H, br d, 9.0) | 4.05 (1 H, d, 11.0) | 4.17 (1 H, d, 11.0) | | C-7" |
| **18** | -* | 4.98 (0.5 H, d, 15.0) | 5.09 (0.5 H, d, 15.0) | | C-9" |
| | -* | 5.03 (1 H, s) | | | C-9" |

[a] Spectra were taken in $CD_3OD$ at 270 MHz. Chemical shifts are ppm relative to internal TMS. Number of protons, multiplicity, and coupling constants in Hz are in parentheses. * This signal could not be observed by overlapping with a solvent signal. † These values are interchangeable.

products were isolated by repeated column and thin layer chromatography followed by HPLC. The major products were the solvolysis products of **3**, racemic dehydroretronecine dimethyl ether (**6**) (ca. 80%) and the necic acid component isolated as lactone acid **7** (ca 80%). The yields of the alkylated nucleosides **12-18** were less than 1% (Table I).

The structures of the alkylated nucleosides **12-18** were determined on the basis of detailed analysis of the UV and the $^1$H NMR spectra coupled with the FAB mass spectra. In particular, the alkylated sites were determined by comparing the UV spectra of the alkylated nucleosides in acidic, neutral, and alkaline solutions with those of the known alkylated nucleosides (Table II). On the other hand, the reacting sites of dehydromonocrotaline (**3**) were determined by comparing the chemical shifts of H-7" and H-9" protons in the alkylated nucleosides with those in dehydroretronecine dimethyl ether (**6**) (Table III): the signals for the H-7" protons in the C-7" $OCH_3$ derivatives of dehydroretronecine (**4**) appear in the region of $\delta$ 4.7–4.8 ppm, while the signals for the H-9" protons in C-9" $OCH_3$ derivatives of **4** appear in the region of $\delta$ 4.0-4.4 ppm. The structures of **12-18** are shown in Table I

Dehydromonocrotaline (**3**) reacted mostly at the C-9" position and alkylated the nucleosides at the nitrogen atoms ; 2'-deoxyadenosine (**8**) at the N-1 and the $N^6$ positions, 2'-deoxyguanosine (**9**) at the N-7 position, thymidine (**10**) at the N-3 position, and 2'-deoxycytidine (**11**) at the N-3 position, respectively. The $N^2$ position in **9** and the N-3 position in **10** were also alkylated with **3** at the C-7" position. No oxygen atom in any nucleoside was alkylated. In contrast, the weaker alkylating agent dehydroretronecine (**4**) reacted always at the C-7" position with nucleosides and nucleotides to give both N- and O-alkylated nucleosides.[3] None of the alkylated nucleosides **12-18** were formed on reaction of dehydroretronecine dimethyl ether (**6**) with the corresponding nucleoside **8-11** under the same conditions as the alkylation with **3**, indicating that alkylated nucleosides **12-18** have come from direct reaction of **3** with the corresponding nucleosides The formation of the N-7 alkylated 2'-deoxyguanosine **14** that may be linked to deprination and strand scission of DNA is noteworthy in view of the hepatotoxicity and the carcinogenicity of monocrotaline (**2**). It is of interest that a metabolite of hepatocarcinogenic aflatoxin $B_1$ also alkylates the N-7 nitrogen of the 2'-deoxyguanosine moiety in oligonucleotides and DNA.[6] Further studies on reaction of dehydromonocrotaline (**3**) with DNA are currently under investigation.

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## References and Notes

(1) Mattocks, A. R. *Chemistry and Toxicology of Pyrrolizidine Alkaloids*; London: Academic Press, 1986.
(2) (a) Mattocks, A. R ; Bird, I. *Toxicicol. Lett.* **1983**, *16*, 1-8; Petry, T. W ; Bowden, G T., Huxtable, R J.; Sipes, I. G. *Cancer Res.* **1984**, *44*, 1505-1509. (b) White, I. N. H.; Mattocks, A. R. *Biochem. J.* **1972**, *18*, 291-297. (c) Reed, R. L.; Ahern, K. G.; Pearson, G. D.; Buhler, D. R *Carcnogenisis* **1988**, *9*, 1355-1361. (d) Weidner, M. F.; Millard, J. T.; Hopkins, P B. *J. Am. Chem Soc.* **1989**, *111*, 9270.
(3) (a) Robertson, K. A. *Cancer Res.* **1982**, *42*, 8-14. (b) Wickramanayake, P P.; Arbogast, B L ; Buhler, D. R.; Deinzer, M. L.; Burlingame, A. L. *J. Am Chem. Soc.* **1985**, *107*, 2485-2488.
(4) (a) Mattocks, A. R *J. Chem Soc. C* **1969**, 1155-1162. (b) Culvenor, C C. J.; Edgar, J. A.; Smith, L. W., Tweeddale, H. J. *Aust J Chem.* **1970**, *23*, 1853-1867. (c) Karchesy, J. J.; Deinzer, M. L. *Heterocycles*, **1981**, 631-634. (d) Karchesy, J. J.; Arbogast, B., Deinzer, M. L. *J Org Chem.* **1987**, *52*, 3867-3872.
(5) Mattocks, A. R.; Jukes, R ; Brown, J. *Toxicon* **1989**, *27*, 561-567.
(6) Gopalakrishnan, S.; Stone, M. P.; Harris, T. M. *J Am. Chem Soc* **1989**, *111*, 7232-7239.

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