Conversion of Linoleic Acid Hydroperoxide to Hydroxy, Keto, Epoxyhydroxy, and Trihydroxy Fatty Acids by Hematin*

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We have carried out a study of the reaction of 13-hydroperoxy-9-cis,11-trans-octadecadienoic acid (linoleic acid hydroperoxide) with hematin. The major products are erythro-13-hydroxy-12,13-epoxy-9-octadecenoic acid, threo-11-hydroxy-12,13-epoxy-9-octadecenoic acid, 9,12,13-trihydroxy-10-octadecenoic acid, 13-keto-9,11-octadecadienoic acid, and 13-hydroxy-9,11-octadecadienoic acid. Several minor products have also been identified, including 9-hydroxy-12,13-epoxyoctadecenoic acid, 11-hydroxy-9,10-epoxy-12-octadecenoic acid, 10-hydroxy-11,12-octadecadienoic acid, and 9-keto-10,12-octadecadienoic acid. Oxygen labeling studies indicate that the observed products arise by at least two pathways. In the major pathway, hematin reduces 13-hydroperoxy-9,11-octadecadienoic acid by one electron to an alkoxyl radical that cyclizes to an adjacent double bond to form an epoxy allylic radical. The allylic radical either couples to the hydroxyl radical coordinated to hematin or diffuses from the solvent cage and couples to O₂, forming a peroxyl radical. In the minor pathway, the hydroperoxide is oxidized by one electron to a 13-peroxyl radical that undergoes β-scission to a pentadienyl radical and O₂. Exchange of hydroperoxide-derived O₂ for dissolved O₂ occurs at this stage followed by coupling of O₂ to either terminus of the pentadienyl radical. Both pathways of hydroperoxide metabolism generate significant quantities of peroxyl radicals that epoxidize the isolated double bonds of dihydroaromatic molecules. The products of hydroperoxide reaction with hematin and the oxygen labeling patterns are very similar to the products of unsaturated fatty acid hydroperoxide metabolism by platelets, aorta, and lung. Our results not only provide a mechanism for the formation of a series of mammalian metabolites of linoleic and arachidonic acids but also offer an estimate of the yield of peroxyl radicals generated during the process.

Hematin (hydroxo-(porphyrinato)iron(III)), the prosthetic group of several hydroperoxide-metabolizing enzymes, catalyzes the epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP-7,8-diol) by fatty acid hydroperoxides (Equation 1). The reaction is potentially important toxicologically because the diol epoxide formed is an extremely reactive and mutagenic metabolite of the environmental carcinogen benzo[a]pyrene (2). Fatty acid hydroperoxides are generated in mammalian tissue by the action of lipoxigenase and cyclooxygenase enzymes of eicosanoid biosynthesis and by lipid peroxidation (3-5). Heme complexes are ubiquitously distributed in mammalian tissue, raising the possibility that peroxyl-dependent oxygenations may contribute to the oxidative metabolism of xenobiotics and endogenous substances.

The mechanism of epoxidation by unsaturated fatty acids and hematin is of considerable interest. We have proposed that hematin reduces the fatty acid hydroperoxide to an alkoxyl radical that adds to the adjacent double bond to form a carbon-centered radical capable of reacting with oxygen to form a peroxyl radical (1). The peroxyl radical, not a heme-oxo complex, is the epoxidizing agent (1). This mechanism predicts that epoxy alcohols are major products of the reaction of fatty acid hydroperoxides with hematin. Preliminary studies confirmed this prediction but indicated an unexpected mechanism for formation of the epoxyl (6). We have performed a more detailed study of the reaction of 13-hydroxylinoleic acid with hematin that catalogs the products generated and provides clues to the electronic changes occurring at the metal center. What emerges is the most complete picture currently available of the interaction of fatty acid hydroperoxides with hematin. It not only provides compelling support for the mechanism we have suggested for epoxidation of BP-7,8-diol but has important implications for the ever-burgeoning field of polysaturated fatty acid oxidation.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**DISCUSSION**

We have carried out an extensive study of the reaction of 13-OOH-18:2 with hematin under conditions analogous to those employed to epoxidize BP-7,8-diol. The major products of the reaction are shown in Fig. 12. Under conditions where 1

* Portions of this paper (including "Experimental Procedures," "Results," Figs. 1-11, and Tables 1-3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-3245, cite the authors, and include a check or money order for $8.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

**REFERENCES**

1. The abbreviations used are: BP-7,8-diol, 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene; 13-OOH-18:2, 13-hydroxy-9-cis,11-trans-octadecadienoic acid (linoleic acid hydroperoxide); BSTFA, bistrimethylsilyltrifluoromethysilane; GC-MS, gas chromatography-mass spectrometry; BHA, butylated hydroxyanisole; Me₃Si, trimethylsilyl; 9-OOH-18:2, 9-hydroperoxy-10-trans,12,cis-octadecadienoic acid; HPLC, high performance liquid chromatography.

2. Portions of this paper (including "Experimental Procedures," "Results," Figs. 1-11, and Tables 1-3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-3245, cite the authors, and include a check or money order for $8.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
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13-OOH-18:2 is not completely consumed, epoxyls (I and II) or epoxyl-derived compounds (V) account for 65% of the identified products whereas the simple reduction and oxidation products, IV and III, account for 35%. The stereochemistry of I-IV has been assigned by comparison of their spectral properties to those reported in the literature. The stereochemistry of the 9-hydroxyl group of V is indeterminate. The zone from which V was purified probably contains isomers epimeric at C-9.

Epoxyols have been reported as products of linoleic acid oxidation in aorta and as products of arachidonic acid oxidation in platelets, aorta, and lung (21–27). Pace-Asciak et al. (27) have shown that the epoxyols produced from arachidonic acid in lung are derived from hydroperoxide intermediates. The hydroxyl groups of the epoxyols are derived primarily from the hydroperoxide oxygen, but a small amount originates in O₂ (26). Similar results have very recently been reported for the metabolism of 12-hydroperoxyeicosatetraenoate by hematin (49). Epoxyols have also been reported as products of hydroperoxy fatty acid metabolism by hemoglobin and by plant and animal lipoxygenases (11, 14). Garssen et al. (11) have shown that the hydroxyl group is derived from the hydroperoxide oxygen in the soybean lipoxygenase-catalyzed reaction. Gardner and co-workers (28–30) have shown that epoxyols are formed by the reaction of 13-OOH-18:2 with Fe²⁺-cysteine, protic acids, or Lewis acids. The Fe²⁺-cysteine-catalyzed reaction is free radical in nature and incorporates O₂ into the hydroxyl group (28). The amounts of I and II are equivalent, indicating no stereoselectivity in the incorporation of O₂. Protic acid-catalyzed epoxyol formation incorporates water into the hydroxyl group (29). When the solvent is methanol, methyl ethers result and the methyl ether corresponding to II predominates over I by 9:1, indicating a high degree of stereoselectivity (29). The source of the hydroxyl oxygen and the stereochemistry of the hematin-catalyzed reaction are clearly different from the acid-catalyzed reaction. Hematin appears to act as a redox catalyst rather than as a Lewis acid. The mechanism we have proposed to explain the reaction is shown in Fig. 13.

The first step in Fig. 13 is one-electron reduction by hematin of 13-OOH-18:2 to an alkoxy radical. The oxidized hematin derivative is a ferryl-hydroxo complex that is electronically analogous to compound II of horseradish peroxidase (31). The direct formation of this ferryl-hydroxo complex as a result of Fe³⁺-porphyrin-peroxide interaction has been suggested by others. George and Irvine (32) originally proposed this complex as an intermediate resulting from the reaction of H₂O₂ with metmyoglobin. Cadenas et al. (33) suggested a similar intermediate in the decomposition of t-butyl hydroperoxide by ferricytochrome c. White et al. (34) and Blake and Coon (35) have proposed that cytochrome P-450 reduces organic hydroperoxides by a similar mechanism. George and Irvine (36) have calculated the free energy for the one-electron reduction of H₂O₂ by metmyoglobin to be +17.9 kcal/mol. If the free energy of the hematin-catalyzed reaction is of similar magnitude, this indicates that the reduction is thermodynamically unfavorable. However, the overall transformation is highly exothermic. Using Benson’s method of group additivity (37, 38) we calculate a ΔG° of -41.6 kcal/mol for the isomerization of 13-OOH-18:2 to epoxyols I or II. This calculation is for molecules in the gas phase but should be a reasonable approximation for the liquid phase as well because the solvation energies of the hydroperoxide and epoxyols should be similar. Exchanging the unstable hydroperoxide oxygen-oxygen bond for a more stable carbon-oxygen σ bond provides the overall driving force for the transformation. Therefore, the thermodynamically unfavorable one-electron reduction of the hydroperoxide by hematin is more than compensated for by the overall exothermicity of the rear-
arrangement and by the exothermicity of the regeneration of hematin from the ferryl-hydroxo complex.

Three products have been identified that appear to arise from 9-OOH-18:2 (Fig. 14). Because this compound is not present in the starting hydroperoxide, it must be formed by isomerization of 13-OOH-18:2. Chan et al. (39) have proposed that unsaturated fatty acid hydroperoxides isomerize via peroxyl radicals generated from hydrogen atom abstraction from the starting hydroperoxide. The peroxyl radicals lose O₂ reversibly and it recouples at either terminus of the pentadienyl radical (Fig. 15). Detailed studies indicate that O₂ loss and readdition are kinetically facile (40). This not only explains the formation of 9-OOH-18:2 but also the exchange of hydroperoxide oxygen with O₂ observed in 13-OOH-18:2 (Table 3). The O₂ formed by β-scission of the peroxyl radical can exchange with dissolved O₂ prior to recombination. This exchange accounts for approximately 60% of the newly formed products of 9-OOH-18:2, and the extent of exchange into III and IV indicates that approximately 8% of 13-OOH-18:2 is metabolized through direct peroxyl radical generation.

Formation of peroxyl radicals from 13-OOH-18:2 is a one-electron oxidation. Although the mechanism of oxidation is unclear, several possibilities exist. A Fenton-type redox cycle (Equations 2 and 3) appears unlikely because it predicts that the yields of alkoxyl- and peroxyl-derived products are the same. This is clearly not the case. Other possibilities include the

\[
\text{ROOH} + \text{Fe}^{2+} \rightarrow \text{ROO}^- + \text{Fe}^{3+} + \text{H}^+ \tag{2}
\]

\[
\text{ROOH} + \text{Fe}^{2+} \rightarrow \text{RO}^- + \text{Fe}^{3+} + \cdot \text{OH} \tag{3}
\]

competition of Reaction 2 with the one-electron reduction pathway or the abstraction of the hydroperoxide hydrogen by an oxidizing agent generated as a result of the one-electron reduction pathway. The oxidizing agent could either be an alkoxyl radical (Equation 4), a peroxyl radical (Equation 5), or a ferryl-hydroxo complex (Equation 6).

\[
\text{ROOH} + \text{R'O}^- \rightarrow \text{ROO}^- + \text{R'O} \tag{4}
\]

\[
\text{ROOH} + \text{R'O}^- \rightarrow \text{ROO}^- + \text{R'O} \tag{5}
\]

\[
\text{ROOH} + \text{Fe}^{2+} \rightarrow \text{ROO}^- + \text{Fe}^{3+} + \text{H}_2\text{O} \tag{6}
\]

The oxidizing agents in Reactions 5 and 6 result from escape from the solvent cage of the radical pair depicted in Fig. 13. R'OO⁻ is the product of O₂ scavenging of the epoxy allylic radical whereas Fe²⁺-OH is the ferryl-hydroxo complex.

The ubiquitous nature of heme complexes, even at relatively low levels, implies that they could be quantitatively important catalysts of hydroperoxide metabolism in mammalian tissue. Indeed, epoxyols and trihydroxy fatty acids were first detected in incubations of arachidonic acid with intact platelets (21). Subsequently, Bryant et al. (19) showed that inclusion of glucose in the platelet suspension medium substantially reduced the yields of triols while increasing the yield of 12-hydroxyeicosatetraenoic acid. They demonstrated that glutathione-glutathione peroxidase action reduces hydroperoxides to hydroxy acids in platelets and that reduction is driven by glucose via the hexose monophosphate shunt. This pathway competes with metal-catalyzed rearrangement of hydroperoxides to epoxides. The level of glutathione or glutathione peroxidase may determine the flux of hydroxy fatty acids through the two pathways. Glutathione-glutathione peroxidase status is a function of genetic, environmental, and nutritional factors and may vary from tissue to tissue. Funk and Powell (41) have recently shown that endogenous linoleic acid is converted to epoxyols and triols by slices of fetal calf aorta suspended in a physiological medium (5.5 mM glucose). This implies that epoxyols are generated under physiological conditions.

The incorporation of O₂ into the hydroxyl groups of epoxyls and triols observed in the present and other studies can only occur through the intermediacy of peroxyl radicals. We estimate that 15–30% of the metabolism of 13-OOH-18:2 to I, II, and V occurs through peroxyl radical intermediates. If a similar percentage obtains in cellular preparations, the concentrations of unsaturated fatty acid-derived epoxyls and triols quantitated in biological samples may provide an estimate of the extent of peroxyl radical generation in vivo. Peroxyl radicals are reactive but have sufficient half-lives (~0.1–10 s) to diffuse considerable distances from the site of their generation (42). Consequently, some of the deleterious effects of unsaturated fatty acid hydroperoxides may arise from peroxyl radicals generated as shown in Fig. 13. The accompanying manuscript (44) indicates that these peroxyl radicals can transfer oxygen to a suitable acceptor. In the case studied, 7,8-dihydroxy-7,8-dihyrobenzo[a]pyrene is epoxidized to a dihydrodihydropoxide, a potent mutagen and carcinogen. This observation suggests that the reaction sequence depicted in Fig. 13 can generate oxidizing agents not only...
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capable of destroying cells but also of transforming them.

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SUPPLEMENTARY MATERIAL TO
CONVERSION OF LINOLEIC ACID HYDROPEROXIDE TO HYDROXY, KETO,
EPONYLTHY, AND TRIHYDROXY FATTY ACIDS BY HEMATIN

Thomas A. Diaz and Lawrence J. Marretti

Experimental Procedures

Materials

Linoleic acid (99%) was obtained from Nutria Prep. [1-14C]Linoleic acid (44 mCi/mmol) and [2-14C]Linoleic acid (36 mCi/mmol) were from New England Nuclear, Boston, MA. Palmitic acid (99%) and stearic acid (99%) were obtained from Calbiochem, San Diego, CA. 2-Butanone (Type IV, 245.9 mmol/liter) and Tween 20 [polyoxyethylene(20) sorbitan monolaurate] were from Sigma Chemical, St. Louis, MO. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was obtained from Sigma Chemical, St. Louis, MO.

Characterization

The metabolism of linoleic hydroperoxide was performed on a Varian Model 5320 instrument. GC-MS was performed on a Hewlett-Packard Model 5890 GC interfaced with a Hewlett-Packard MS-series mass spectrometer. Sample systems were eluted through 6 m, 0.18 diam, glass columns. Capillary columns were determined with deuterated and acetylated standards. Identification of peaks was based on retention time and mass spectra matching the mass spectra of the standard.

Preparation of [1-14C]linoleic and [2-14C]linoleic acids

[1-14C]linoleic acid was prepared from linoleic acid (Stahler, 4.8 Ci/mmol) as described by Fisk et al. (1971). Separation of linoleic acid from hydroxy and keto fatty acids was achieved by passage through a column of Amberlite XAD-2 (20 g). After washing the column with methanol, the soluble fraction was eluted with 0.1 N HCl and subsequently washed with water. The fractions were concentrated by distillation under reduced pressure. The extract was evaporated to dryness and the residue was dissolved in 250 mL of 0.1 N HCl and subjected to chromatography on a silica gel column. The column was eluted with a gradient of methanol-water (from 0% to 100% methanol over 20 min) and fractions were collected. After evaporation, the residue was dissolved in 1 N HCl and subjected to radioactivity measurements. A mixture of the two fractions was used in all experiments. Rates of metabolism were determined by measurement of radioactivity remaining in the supernatant of the reaction mixture after extraction with ethyl ether (method of Poole). The mixture was then counted in a liquid scintillation counter.

Conversion of linoleic acid to its hydroperoxide

The metabolism of linoleic hydroperoxide was determined after reduction, addition, digestion, and GC-MS analysis, as described above. The reaction mixture was diluted with water and extracted with ethyl ether. The solvent was removed under reduced pressure. The extract was then subjected to radioactivity measurements. A mixture of the two fractions was used in all experiments. Rates of metabolism were determined by measurement of radioactivity remaining in the supernatant of the reaction mixture after extraction with ethyl ether (method of Poole). The mixture was then counted in a liquid scintillation counter.

Conversion of [1-14C]linoleic acid to products by hemat in

Hematin was added to a solution of linoleic acid (0.1 M) in phosphate buffer (pH 7.10), and after 15 min the reaction was initiated by the addition of NADPH. After 15 min, the mixture was extracted with ethyl ether, and the solvent was removed under reduced pressure. The extract was then subjected to radioactivity measurements. A mixture of the two fractions was used in all experiments. Rates of metabolism were determined by measurement of radioactivity remaining in the supernatant of the reaction mixture after extraction with ethyl ether (method of Poole). The mixture was then counted in a liquid scintillation counter.

Summary of the reaction of Hematin with linoleic hydroperoxide

The reaction of Hematin with linoleic hydroperoxide was performed at 37°C in phosphate buffer (pH 7.10) as described above. Hematin was added to a solution of linoleic acid (0.1 M) in phosphate buffer (pH 7.10), and after 15 min the reaction was initiated by the addition of NADPH. After 15 min, the mixture was extracted with ethyl ether, and the solvent was removed under reduced pressure. The extract was then subjected to radioactivity measurements. A mixture of the two fractions was used in all experiments. Rates of metabolism were determined by measurement of radioactivity remaining in the supernatant of the reaction mixture after extraction with ethyl ether (method of Poole). The mixture was then counted in a liquid scintillation counter.

Identification of the major products of the reaction of Hematin with [1-14C]-linoleic acid

The products of the reaction of Hematin with [1-14C]-linoleic acid were fractionated by thin-layer chromatography on Silica Gel G. The reaction mixture was extracted with ethyl ether, and the solvent was removed under reduced pressure. The extract was then subjected to radioactivity measurements. A mixture of the two fractions was used in all experiments. Rates of metabolism were determined by measurement of radioactivity remaining in the supernatant of the reaction mixture after extraction with ethyl ether (method of Poole). The mixture was then counted in a liquid scintillation counter.

Figure 1: HPLC separation of products of reaction of Hematin with [1-14C]-linoleic acid

The products of the reaction of Hematin with [1-14C]-linoleic acid were fractionated by thin-layer chromatography on Silica Gel G. The reaction mixture was extracted with ethyl ether, and the solvent was removed under reduced pressure. The extract was then subjected to radioactivity measurements. A mixture of the two fractions was used in all experiments. Rates of metabolism were determined by measurement of radioactivity remaining in the supernatant of the reaction mixture after extraction with ethyl ether (method of Poole). The mixture was then counted in a liquid scintillation counter.
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Table 1. Yields of Major Products Resulting from the Conversion of 12-OH-18:2 by Hematin

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield (%) and Derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11.1</td>
</tr>
<tr>
<td>II</td>
<td>11.1</td>
</tr>
<tr>
<td>III</td>
<td>16.3</td>
</tr>
<tr>
<td>IV</td>
<td>5.2</td>
</tr>
<tr>
<td>V</td>
<td>14.2</td>
</tr>
<tr>
<td>13-OH</td>
<td>10.4</td>
</tr>
<tr>
<td>unidentified</td>
<td>17.5</td>
</tr>
<tr>
<td>nonidentified</td>
<td>18.2</td>
</tr>
</tbody>
</table>

Identification and Quantitation of Minor Reaction Products

During gas chromatography of Zones II and C to locate individual samples of the major reaction products, a number of minor peaks were noted (see Figure 3). To try to identify these minor peaks, the gas chromatography was repeated using different GC-MS operating conditions to identify the major reaction products. The products were extracted, separated, and subjected to GC-MS analysis without further chromatographic purification. This helped to prevent loss of minor products because the initial separation of products into zones was performed at neutral pH. Each zone was then subjected to complete GC-MS analysis of all major and minor derivatives present in the GC output base line.

The total ion profile of Zone B (the area containing epoxy-hydroxy products) and Zone II is shown in Figure 6. The major peak (peaking at 9.63 mins) contains derivatives I and II.

Zones II and V are indicated by the arrow in Figure 6. Zone V (9.13 mins) contains derivatives II and V, which are not present in Zone II. The total ion profile of Zone V is shown in Figure 7. The major peaks (peaking at 10.08 mins) correspond to derivatives II and V. The peaks at 9.13 mins are not clearly distinct, and since Zone V contains only one major peak, it is not possible to discern the exact structure of these peaks.
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The total ion profile of Zone C (the zone containing II and III) is shown in Figure 9. The two major peaks seen at 1.0 and 1.15 min were identified as YV and YII, respectively, whereas the peak at 1.5 min was identified as a myristic contaminant. The small peak at 2.5 min (product YVIII) gave the mass spectrum shown in Figure 10. A small peak at 3.0 min (product YX) gave the mass spectrum shown in Figure 11. This peak gave a molecular ion at m/z 380 and other ions of high intensity at m/z 284 (C18H180), 222 (C16H16O), and 116 (C8H14O).

Figure 9. Total ion profile of eluted Zone C (Figure 1) determined by GC-MS.

Figure 10. Mass spectrum of derivative product YVIII.

Figure 11. Mass spectrum of derivative product YX.

Table 2. Yield of Methyl Products Resulting from the Conversion of Linoleate Hydroperoxide

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YI</td>
<td>3-4</td>
</tr>
<tr>
<td>YII</td>
<td>1</td>
</tr>
<tr>
<td>YVIII</td>
<td>2-3</td>
</tr>
<tr>
<td>YIX</td>
<td>3-0</td>
</tr>
</tbody>
</table>

Table 3. Incorporation of NO_2 into Products of Linoleate Hydroperoxide

<table>
<thead>
<tr>
<th>Product</th>
<th>Alkyl % Exposed NO_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>12.4</td>
</tr>
<tr>
<td>YII</td>
<td>15.5</td>
</tr>
<tr>
<td>YIII</td>
<td>68.7</td>
</tr>
<tr>
<td>YX</td>
<td>58.2</td>
</tr>
</tbody>
</table>

Extraction of hydrogen peroxide and atmospheric oxygen

The intracellular environment of the yeast was determined for the formation of products YII and YVII (Table 1) and product YVIII (Table 2). The yield of product YII was determined in each experiment with hematin under an NO_2 atmosphere whereas product YVIII was determined under an NO_2 atmosphere in the second experiment. The yield was determined by assaying the absorbance at 280 nm and 350 nm, respectively, of the biotic reaction products. The yield was determined by assaying the absorbance at 280 nm and 350 nm, respectively, of the biotic reaction products.

The identification of products YII and YVIII was in agreement with the mass spectra of these compounds. The mass spectra of these compounds are shown in Figures 9 and 10. The mass spectra of these compounds are shown in Figures 9 and 10.

Table 3 shows the data for the NO_2 incorporation into the products of products YII and YVIII (Table 1). The NO_2 incorporation into the products YII and YVIII (Table 1) is shown in Figure 11. The NO_2 incorporation into the products YII and YVIII (Table 1) is shown in Figure 11.

(a) Structures of products are given in the discussion. (b) (ii) (ii) and (ii) (ii) are defined as NO_2 derivatives in Table 1. (iii) and (iii) are defined as NO_2 derivatives in Table 1.