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## INHIBITION OF LIPID PEROXIDATION MEDIATED BY INDOLIZINES

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**Abstract:** Esters, ethers, carbonates and carbamates of 1-indolizinols and azaindolizinols exhibit a profound inhibition of lipid peroxidation *in vitro*. The antioxidants were prepared by cyclization of pyridines and diazines with diphenylcyclopropenone followed by introduction of the *O*-substituent.

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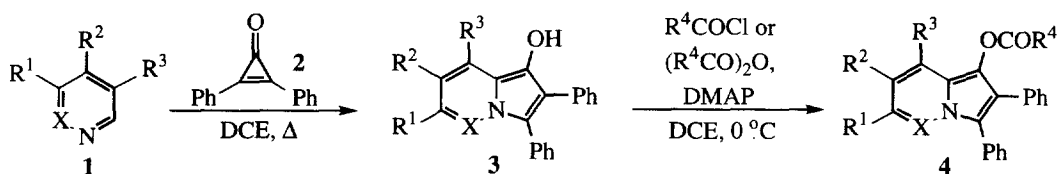
Compounds with antioxidant / radical scavenging properties may have great therapeutic potential because free radicals have been linked to several major diseases. Selected examples are cancer, Parkinson's disease, Alzheimer's disease, stroke, heart infarction and rheumatoid arthritis.<sup>1</sup> It is reported that several 1-indolizinols are easily oxidized to stable free radicals.<sup>2</sup> We therefore envisaged that *O*-protected indolizinols may act as stable precursors for highly potent antioxidants.<sup>3</sup> In this communication, we wish to report examples of indolizine derivatives which strongly inhibit lipid peroxidation *in vitro*, probably by an electron donation mechanism.

Among the methods available for indolizine synthesis,<sup>4</sup> relatively few are known for facile preparation of indolizinols. We chose to prepare the indolizinyll esters **4** by reaction of pyridines or diazines **1** with diphenylcyclopropenone **2** followed by acylation of the intermediate indolizinols<sup>5,6</sup> (Scheme 1).

The ability of the indolizinyll- and azaindolizinyll esters to inhibit lipid peroxidation *in vitro* was examined and the results are summarized in Table 1.<sup>7,8</sup> Several of the acetates inhibited lipid peroxidation strongly, while the fatty acid ester **4c** (Table 1, Entry 3) exhibited only weak activity. Electron withdrawing substituents in the 6-membered ring appear to increase the antioxidant activity somewhat compared to the unsubstituted compound **4a** (Table 1, Entry 1). Also the azaindolizine **4g** prepared from pyridazine (Table 1, Entry 7) was highly active, but the benzofused analog **4h** was a poor antioxidant (Table 1, Entry 8). For comparison, the antioxidants rutin and cyanidine have IC<sub>50</sub> values of 10 and 27 μM respectively, in the same hepatic microsomal Fe/ascorbate system.<sup>9</sup>

The indolizine esters described herein, may inhibit lipid peroxidation by several mechanisms. One alternative is that the esters are hydrolyzed to indolizinols **3** which then act as hydrogen atom donors towards radicals generated in the test medium, while forming neutral indolizinyll radicals as the by product. Alternatively, the esters themselves may be oxidized to stable cation radicals while donating electrons and thereby terminate the lipid peroxidation radical chain reactions. Radical cations have been generated from α-tocopherol, its acetate and methyl ether.<sup>10</sup> Yet another possibility is that the indolizines form complexes with Fe<sup>2+</sup> and thus inhibit the onset of the lipid peroxidation.

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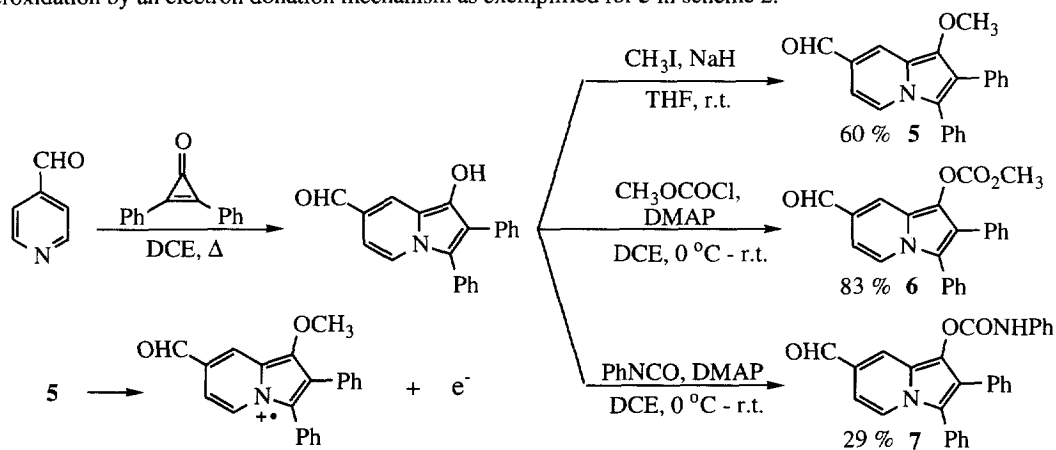
Scheme 1

Table 1. Inhibition of lipid peroxidation *in vitro* mediated by the esters 4.

Entry	Compound	-X-	-R <sup>1</sup>	-R <sup>2</sup>	-R <sup>3</sup>	-R <sup>4</sup>	IC <sub>50</sub> values <sup>a</sup>
1	<b>4a</b>	-CH-	-H	-H	-H	-CH <sub>3</sub>	11.8
2	<b>4b</b>	-CH-	-H	-CHO	-H	-CH <sub>3</sub>	2.9
3	<b>4c</b>	-CH-	-H	-CHO	-H	-(CH <sub>2</sub> ) <sub>14</sub> CH <sub>3</sub>	9 % at 100 μM <sup>b,c</sup>
4	<b>4d</b>	-CH-	-H	-COCH <sub>3</sub>	-H	-CH <sub>3</sub>	5.3
5	<b>4e</b>	-CH-	-COCH <sub>3</sub>	-H	-H	-CH <sub>3</sub>	3.5
6	<b>4f</b>	-CH-	-H	-COPh	-H	-CH <sub>3</sub>	2.5
7	<b>4g</b>	-N-	-H	-H	-H	-CH <sub>3</sub>	3.1
8	<b>4h</b>	-N-	-H	-C <sub>4</sub> H <sub>4</sub> -	-H	-CH <sub>3</sub>	28 % at 100 μM <sup>b,c</sup>

<sup>a</sup> IC<sub>50</sub> (μM) is the concentration which causes 50 % inhibition of lipid peroxidation after 30 min. The values are given as the mean of 3 separate experiments and the accuracy of the data is within 25 %. <sup>b</sup> When no IC<sub>50</sub> value could be determined, the % inhibition at 100 μM conc. is reported. <sup>c</sup> The high IC<sub>50</sub> values of **4c** and **4h** might result from different physical properties (higher lipophilicity) relative to the other substances.

In order to shed some light on the mechanism of antioxidant activity, we prepared the methyl ether **5**,<sup>11</sup> carbonate **6**<sup>12</sup> and carbamate **7**<sup>12</sup> (Scheme 2) and compared their ability to inhibit lipid peroxidation with that of the acetate **4b** (Table 2). Regardless of the identity of the *O*-substituent, the compounds examined here exhibited comparable activity in the test system. These results supported the hypothesis that the indolizines themselves act as antioxidants, especially cleavage of the methyl ether **5** to the unprotected indolizinol is highly unlikely. Cyclic voltammetry of compounds **4b**, **5** and **6** showed reversible oxidations to the corresponding radical cations with E<sup>0</sup> (vs Fc/Fc<sup>+</sup>) in the range of 0.4–0.7 V (Table 2). These findings indicate that the indolizines *may* inhibit lipid peroxidation by an electron donation mechanism as exemplified for **5** in scheme 2.



Scheme 2

**Table 2.** Antioxidant and red-ox properties of indoliziny ester, ether, carbonate and carbamate.

Entry	Compound	IC <sub>50</sub> values <sup>a,d</sup>	E° vs Fc/Fc <sup>+</sup> (V) <sup>b,d</sup>
1	Acetate <b>4b</b>	2.9	0.64
2	Ether <b>5</b>	4.2	0.38
3	Carbonate <b>6</b>	2.6	0.65
4	Carbamate <b>7</b>	3.2	— <sup>c</sup>

<sup>a</sup> IC<sub>50</sub> (μM) is the concentration which causes 50 % inhibition of lipid peroxidation after 30 min. The values are given as the mean of 3 separate experiments and the accuracy of the data is within 25 %. <sup>b</sup> 1.0 mM solutions in MeCN containing 0.1 M Bu<sub>4</sub>NPF<sub>6</sub>. <sup>c</sup> Not determined. <sup>d</sup> No clear correlation between IC<sub>50</sub> and E° has been found so far.

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#### References and Notes

- See for instance: (a) Halliwell, B. *Drugs* **1991**, 42, 569; (b) Rice-Evans, C. A.; Diplock, A. T. *Free Radical Biol. Med.* **1993**, 15, 77; (c) Bast, A. *Pharmacochem. Libr.* **1993**, 20, 159; (d) Halliwell, B. *Chem. Edu.* **1995**, 123.
- (a) Wadsworth, D. H.; Bender, S. L.; Smith, D. L.; Luss, H. R.; Weidner, C. H. *J. Org. Chem.* **1986**, 51, 4639; (b) Wadsworth, D. H.; Weidner, C. H.; Bender, S. L.; Nuttall, R. H.; Luss, H. R. *J. Org. Chem.* **1989**, 54, 3652; (c) Weidner, C. H.; Wadsworth, D. H.; Bender, S. L.; Beltman, D. J. *J. Org. Chem.* **1989**, 54, 3660; (d) Weidner, C. H.; Michaels, F. M.; Beltman, D. J.; Montgomery, C. J.; Wadsworth, D. H.; Briggs, B. T.; Picone, M. L. *J. Org. Chem.* **1991**, 56, 5594.
- Rise, F.; Wikström, H.; Ugland, S.; Dijkstra, D.; Gundersen, L.-L.; De Boer, P.; Bast, A.; Haenen, G.; Antonsen, Ø.; Liao, Y.; Nasir, A. I. *PCT Int. Appl. WO 96 21,662*, **1996** (*Chem. Abstr.* **1996**, 125 195681c).
- See for instance: (a) Uchida, T.; Matsumoto, K. *Synthesis* **1976**, 209; (b) Joule, J. A.; Mills, K.; Smith, G. F. *Heterocyclic Chemistry*, 3rd Ed., pp 434-441; Chapman & Hall; London, 1995.
- Compounds **4a**, **4b**, and **4d** were prepared essentially as described in ref. 2a; compounds **4g** and **4h** were prepared according to ref. 2d.
- General procedure for the preparation of unknown indolizine esters, the synthesis of compound **4e** is representative: A mixture of diphenylcyclopropenone (0.5 mmol) and 3-acetylpyridine (0.5 mmol) in dry dichloroethane (30 ml) was refluxed under N<sub>2</sub> for 24 h and cooled to 0 °C before *N,N*-dimethylamino-pyridine (1.3 mmol) and acetic acid anhydride (1.2 mmol) dissolved in dichloroethane (10 ml) was added. The resulting mixture was stirred at 0 °C for 1 h and at room temperature for 1 h, diluted with CHCl<sub>3</sub> (60 ml) and washed with sat. aq. CuSO<sub>4</sub> (4×30 ml), sat. aq. NaHCO<sub>3</sub> (2×30 ml) and brine (30 ml). The dried (MgSO<sub>4</sub>) solution was evaporated and the product purified by flash chromatography to give compound **4e**; yield 43 %. M.p. 173-176 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz): δ 2.31 (3H, s), 2.47 (3H, s), 6.3-6.5 (1H, dd), 7.20-7.40 (12H, m), 8.11 (1H, s). The compounds **4c** and **4f** were prepared by the same procedure except that palmitoyl chloride was used in the synthesis of **4c**; yields 48 % of **4c** and 60 % of **4f**.

7. Male Wistar rats (200–220 g) were killed by decapitation. Livers were removed and homogenized (1:2 w/v) in ice cold phosphate buffer (50 mM, pH>7.4) containing 0.1 mM EDTA. The homogenate was centrifuged at 10,000 g (20 min) and 100,000 g (60 min). The microsomal pellet was resuspended in the phosphate buffer (2 g liver/ml) and stored at -80 °C. Before use the microsomes were thawed and diluted at least 5 fold with ice-cold Tris-HCl buffer (50 mM, pH 7.4) containing 150 mM KCl, and washed twice with centrifugation at 115,000 g (45 min). The pellet was resuspended, the final concentration in the assay was microsomes derived from 1/8 g liver per ml. The microsomes were boiled at 100 °C for 90 seconds in order to avoid enzymatic interference. Microsomal lipid peroxidation was induced by the combination of ascorbic acid (0.1 mM) and FeSO<sub>4</sub> (10 µM). Lipid peroxidation was determined by measuring the thiobarbituric acid (TBA) reactive material. An aliquot of the incubation mixture was mixed with ice-cold TBA-trichloroacetic acid-HCl-butylhydroxytoluene solution (1 ml). After heating (15 min, 80 °C) and centrifugation (15 min, 5,000 g) the absorbance at 535 versus 600 nm was determined, which was used as a measure of lipid peroxidation. The TBA-trichloroacetic acid-HCl solution was prepared by dissolving 4.16 mg TBA/ml trichloroacetic acid-HCl solution (16.8 % w/v in 0.125 M HCl). To 10 ml of the TBA-trichloroacetic acid-HCl solution 1 ml butylhydroxytoluene (1.5 mg/ml ethanol) was added. The IC<sub>50</sub> values were calculated from the concentrations of the compounds that gave a protection just above and below 50 % compared to the control incubation without the compound.
8. Haenen, G. R. M. M.; Bast, A. *Fedn. Eur. Biochem. Soc. Lett.* **1983**, 159, 24.
9. van Acker, S. A. B. E.; van den Berg, D.-J.; Tromp, M. N. J. L.; Griffioen, D. H.; van Bennekom, W. P.; van der Vijgh, W. J. F.; Bast, A. *Free Radical Biol. Med.* **1996**, 20, 331.
10. (a) Craw, M. T.; Depew, M. C. *Rev. Chem. Intermed.* **1985**, 6, 1; (b) Sur, S.; Colpa, J. P. *Chem. Phys. Lett.* **1986**, 127, 577; (c) Depew, M. C.; Craw, M. T.; MacCormick, K.; Wan, J. K. S. *Photochem. Photobiol. B* **1987**, 229; (d) Ishar, M. P. S.; Kaur, R.; Kaur, G.; Gandhi, R. P. *Ind. J. Chem.* **1996**, 35B, 642.
11. A mixture of diphenylcyclopropanone (0.5 mmol) and 4-pyridinecarbaldehyde (0.5 mmol) in dry DCE (30 ml) was refluxed under N<sub>2</sub> for 24 h, cooled and evaporated. The residue was dissolved in dry THF (30 ml) and sodium hydride (1.1 mmol) and iodomethane (1.1 mmol) were added. The resulting mixture was stirred at ambient temperature under N<sub>2</sub> for 24 h and evaporated. Ether (100 ml) was added to the residue and the solution was washed with water (2×30 ml), dried (MgSO<sub>4</sub>) and evaporated. The product was isolated by flash chromatography on silica gel eluting with EtOAc-hexane (1:7); yield 60 %. M.p. 157–159 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz): δ 3.73 (3H, s), 6.77 (1H, dd), 7.1–7.4 (10H, M), 7.75 (1H, d), 7.92 (1H, s), 9.75 (1H, s).
12. A mixture of diphenylcyclopropanone (0.5 mmol) and 4-pyridinecarbaldehyde (0.5 mmol) in dry DCE (30 ml) was refluxed under N<sub>2</sub> for 24 h and cooled to 0 °C before *N,N*-dimethylaminopyridine (1.3 mmol) and methyl chloroformate or phenylisocyanate (2.0 mmol) were added. The resulting mixture was stirred at 0 °C for 10 h and at room temperature for 14 h and evaporated and the product was isolated by flash chromatography on silica gel. **6**: Yield 83 %. M.p. 72–74 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz): δ 3.92 (3H, s), 7.02 (1H, dd), 7.0–7.5 (10H, m), 7.93 (1H, dd), 7.99 (1H, s), 9.86 (1H, s). **7**: Yield 29 %. M.p. 130–133 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz): δ 6.91 (1H, s), 7.2–7.4 (16H, m), 7.83 (1H, s), 7.89 (1H, d), 9.72 (1H, s).