

over 99 %; it is much higher than that of the same ester synthesized through step-wise coupling of small deuterated units.<sup>3</sup>

Full details of this work will be published later.

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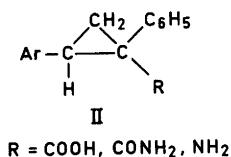
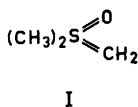
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## 1,2-Diarylcyclopropane Derivatives

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Recently, Corey and Chaykovsky<sup>1</sup> observed that dimethyloxosulfonium methylide (I) generated from the readily accessible trimethylsulfoxonium iodide<sup>2</sup> by proton transfer to a strong base reacted with a conjugated carbonyl compound receptive to Michael addition to give a cyclopropane derivative. The application of this reagent was extended to alpha-substituted stilbenes with a view to obtain 1,2-diarylcyclopropane derivatives (II) which might possess useful pharmacological properties.



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Ethyl  $\alpha$ -phenylcinnamate obtained by esterification of *trans*  $\alpha$ -phenylcinnamic acid reacted with the ylide I to form ethyl 1,2-diphenylcyclopropanecarboxylate in 78.6 % yield; hydrolysis of the ester with ethanolic KOH yielded a mixture of two isomeric acids which were separated by fractional crystallization from ethanol. The two isomeric acids were subjected to modified Curtius reaction<sup>3</sup> to obtain crude isocyanates which were hydrolysed by suspending in 15 % aq. KOH and letting the mixture stand at room temperature for 4–7 days with occasional shaking. The amines were isolated as the hydrochlorides.

In the case of 1-phenyl-2-(*p*-anisyl)- and 1-phenyl-2-(*p*-chlorophenyl)-cyclopropanecarboxylic acids it was found advantageous to convert the crude azides into benzylurethans which smoothly underwent catalytic hydrogenation to give the amines.

In order to study the effect of chain length,  $\alpha$ -phenylcinnamionitrile was reacted with the ylide I to obtain 1,2-diphenylcyclopropylcyanide; LiAlH<sub>4</sub> reduction of the nitrile gave 1,2-diphenylcyclopropylmethylamine isolated as the hydrochloride. Jaz and Weiler<sup>4</sup> have recently reported the formation of cyclopropane derivatives by the addition of diazomethane to  $\alpha$ -cyano-stilbenes.

Preliminary pharmacological testing of the compounds listed in Table I indicated that they were quite toxic and did not exhibit significant pharmacological effects.\*\*

*Experimental.* Microanalyses were carried out by W. Egger and G. Cornali of these laboratories.

The procedure used for the preparation of 1,2-diarylcyclopropanecarboxylic acids is illustrated by the following example.

*1,2-Diphenylcyclopropanecarboxylic acid.* To a stirred solution of trimethylsulfoxonium iodide (0.15 mole) in 225 ml of dimethylsulfoxide in nitrogen atmosphere sodium hydride (0.15 mole, 46 % suspension in mineral oil) was added in small portions. When hydrogen evolution was complete, a solution of ethyl  $\alpha$ -phenylcinnamate (0.12 mole) in 75 ml of dimethylsulfoxide was added dropwise. The reaction mixture was stirred for 1 h after the addition was over and was then placed in a water bath maintained at 55–60° for 1.5 h. It was poured into ice water and the mixture

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Table 1. 1,2-Diarylcyclopropane derivatives

Ar	R	M.p., °C	Formula	C		H		N	
				Calc.	Found	Calc.	Found	Calc.	Found
1. C <sub>6</sub> H <sub>5</sub>	COOH	145–150°	C <sub>16</sub> H <sub>14</sub> O <sub>2</sub>	80.64	80.63	5.92	5.88	—	—
2. C <sub>6</sub> H <sub>5</sub>	COOH	220–222°	C <sub>16</sub> H <sub>14</sub> O <sub>2</sub>	—	—	—	—	—	—
3. C <sub>6</sub> H <sub>5</sub>	CONH <sub>2</sub>	112–120° <sup>a</sup>	C <sub>16</sub> H <sub>16</sub> NO	80.98	81.02	6.37	6.46	5.90	5.98
4. C <sub>6</sub> H <sub>5</sub>	CONH <sub>2</sub>	167.5–169.5 <sup>b</sup>	C <sub>16</sub> H <sub>16</sub> NO	80.98	80.95	6.37	6.37	5.90	5.83
5. C <sub>6</sub> H <sub>5</sub>	NH <sub>2</sub> ·HCl	154–155.5°	C <sub>16</sub> H <sub>16</sub> ClN	73.31	73.34	6.56	6.49	5.70	5.59
6. C <sub>6</sub> H <sub>5</sub>	NH <sub>2</sub> ·HCl	180–183°	C <sub>15</sub> H <sub>16</sub> ClN	73.31	73.38	6.56	6.69	5.70	5.65
7. C <sub>6</sub> H <sub>5</sub>	CN	57–59.5°	C <sub>16</sub> H <sub>15</sub> N	87.64	87.59	5.98	6.14	—	—
8. C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub> NH <sub>2</sub> ·HCl	227–230°	C <sub>16</sub> H <sub>18</sub> ClN	73.97	73.99	6.98	7.12	5.39	5.34
9. 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	COOH	156–188°	C <sub>17</sub> H <sub>16</sub> O <sub>3</sub>	76.10	76.24	6.01	5.88	—	—
10. 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	CONH <sub>2</sub>	146–150.5°	C <sub>17</sub> H <sub>17</sub> NO <sub>2</sub>	76.38	76.44	6.41	6.51	5.24	5.21
11. 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	NHCOOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	106–106.5°	C <sub>24</sub> H <sub>23</sub> NO <sub>3</sub>	77.19	77.05	6.21	6.28	3.75	3.81
12. 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	NH <sub>2</sub> ·HCl	148–151°	C <sub>16</sub> H <sub>18</sub> ClNO	69.68	69.66	6.58	6.61	5.08	5.17
13. 4-ClC <sub>6</sub> H <sub>4</sub>	COOH	169–171°	C <sub>16</sub> H <sub>13</sub> ClO <sub>2</sub>	70.46	70.32	4.81	4.77	—	—
14. 4-ClC <sub>6</sub> H <sub>4</sub>	CONH <sub>2</sub>	160–163°	C <sub>16</sub> H <sub>13</sub> ClNO	70.71	70.65	5.19	5.29	5.16	4.99
15. 4-ClC <sub>6</sub> H <sub>4</sub>	NHCOOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	130.5–131°	C <sub>16</sub> H <sub>13</sub> ClNO <sub>2</sub>	73.10	72.80	5.34	5.15	3.71	3.69
16. 4-ClC <sub>6</sub> H <sub>4</sub>	NH <sub>2</sub> ·HCl	156–162°	C <sub>15</sub> H <sub>13</sub> Cl <sub>2</sub> N	64.30	64.31	5.40	5.45	5.00	4.90
17. 3,4-OCH <sub>2</sub> OC <sub>6</sub> H <sub>3</sub>	COOH	222–225°(decomp.)	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub>	72.33	72.30	4.99	5.05	—	—
18. 3,4-OCH <sub>2</sub> OC <sub>6</sub> H <sub>3</sub>	CONH <sub>2</sub>	160–165°	C <sub>17</sub> H <sub>15</sub> NO <sub>3</sub>	72.58	72.58	5.38	5.43	4.98	4.99
19. 3,4-OCH <sub>2</sub> OC <sub>6</sub> H <sub>3</sub>	NHCOOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	114.5–115.5°	C <sub>24</sub> H <sub>21</sub> NO <sub>4</sub>	74.40	74.22	5.46	5.38	3.62	3.50

<sup>a</sup> Obtained from compd. No. 1. <sup>b</sup> Obtained from compd. No. 2.

was extracted twice with ether. The ether was removed under reduced pressure and the residual liquid was taken up in acetonitrile. The acetonitrile solution was washed with petroleum ether (b.p. 30–60°) to remove mineral oil and then dried over anhydrous magnesium sulfate. After removing the solvent the residual liquid was distilled to obtain ethyl 1,2-diphenylcyclopropanecarboxylate, b.p. 130–135°/0.1 mm Hg, in 78.6 % yield.

The foregoing ester was dissolved in 200 ml of ethanol and aqueous potassium hydroxide (0.2 mole dissolved in 25 ml of water) was added to it. The mixture was refluxed for 15 h. The ethanol was distilled off under reduced pressure, the residue was diluted with water and the mixture was extracted with ether to remove unhydrolysed material. The aqueous solution was made acidic and the resulting precipitate was filtered. Fractional crystallization from ethanol yielded two acids: (i) m.p. 145–150°; yield, 12.65 g. (ii) m.p. 220–222°; yield, 6.2 g.

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## Induced Heterogeneity of Lactoperoxidase

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An earlier study,<sup>1</sup> employing chromatography on Sephadex-DEAE, demonstrated several enzymically active fractions of peroxidase prepared from cow's milk. The existence of multiple forms of LPO\* was later confirmed by the separa-

tion of this enzyme into six zones by means of electrophoresis in a stable pH-gradient<sup>2</sup> and into five zones by disc electrophoresis.<sup>3</sup> Morrison and Hultquist<sup>4</sup> have suggested that the two earlier known forms of LPO, A and B, might be the result of the proteolytic activity of rennin, which is used in the preparation procedure, or due to genetic differences among the cows. However, the heterogeneity pattern was not affected by the omission of rennin from the preparation procedure,<sup>1,2</sup> neither did milk from a single cow yield a homogeneous enzyme.<sup>1</sup> The ratio between the light absorption at the Soret-band, 412 m $\mu$ , and at 280 m $\mu$  is different for the subfractions. The highest value is obtained for that fraction which has the highest isoelectric point, fraction 1. Upon storage in 80 % saturated ammonium sulfate the light absorption ratio of chromatographically pure fraction 1 decreased to a value resembling that of fraction 2. This observation suggested a conversion of fraction 1 into fraction 2.<sup>1</sup> The purpose of the present investigation is to determine, if such a conversion can be demonstrated, using disc electrophoresis.

*Materials and methods.* LPO was prepared as described previously.<sup>1</sup> Disc electrophoresis was carried out according to Ornstein and Davis<sup>5</sup> and Broome<sup>6</sup> with some modifications.<sup>3</sup> "Preparative" disc electrophoresis was performed in glass tubes with an internal diameter of 0.9 cm compared to 0.5 cm for the analytical separation. All chemicals used were of analytical grade.

*Results and discussion.* LPO was separated into three major and two minor zones by disc electrophoresis in polyacrylamide gel.<sup>3</sup> These zones are designated, from cathode to anode, LPO 1 – LPO 5. The main fraction, LPO 1, corresponding to the fraction with the highest isoelectric point,<sup>2</sup> was collected from "preparative" runs by cutting out the zone from the polyacrylamide. The gel was homogenized in a Potter-Elvehjem homogenizer and then extracted with 0.06 M potassium acetate buffer, pH 6.5. The enzyme thus obtained, which moved as a single zone upon repeated electrophoresis, was dialysed at room temperature for 36 h against 40 % saturated ammonium sulfate, adjusted to pH 9.4 with ammonium hydroxide. After an additional dialysis against 0.06 M potassium acetate buffer, pH 6.5, the enzyme was analyzed by disc electrophoresis. The peroxidase was now heterogeneous and was composed of the original frac-

\* Abbreviation: LPO, lactoperoxidase