(92), 28 (15); exact mass calcd for $C_{13}H_{15}NO_4S$ 281.0722, found 281.0732.

Hydrogenation of Ene Product 32. To a solution of 0.036 g (0.12 mmol) of alkene **32** in 10 mL of ethyl acetate was added 0.010 g of 10% Pd/C. The mixture was stirred under 1 atm of hydrogen for 12 h, was filtered through Celite, and was concentrated in vacuo to provide 0.036 g (100%) of white crystalline **34**: mp 154 °C (recrystallized from ethyl acetate–hexane); IR (film) 3275, 2960, 1740, 1350, 1170, 815, 675 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 7.76 (2 H, d, J = 8.3 Hz), 7.31 (2 H, d, J = 8.5 Hz), 5.55 (1 H, d, J = 5.0 Hz), 4.33 (1 H, m), 4.24 (1 H, m), 4.05 (1 H, dd, J = 6.8, 5.0 Hz), 2.47 (1 H, m), 2.42 (3 H, s), 2.11 (1 H, m), 1.80 (1 H, m), 1.66 (1 H, m), 1.36 (1 H, m), 1.12 (1 H, m), 0.92 (1 H, m), 0.87 (3 H, t, J = 7.3 Hz); mass spectrum, m/z (relative intensity) 239 (26), 210 (78), 156 (70), 155 (48), 91 (100), 84 (22), 65 (29), 55 (28), 41 (25), 28 (46).

Preparation of Diels–Alder Adduct 45. Benzamide (0.500 g, 4.13 mmol) was added to a stirred solution of glyoxylate **25c** (0.481 g, 3.75 mmol) in 15 mL of dry acetone. The solution was stirred at room temperature for 62 h. Removal of the solvent in vacuo gave a solid which was purified by preparative TLC (ethyl acetate–hexane, 1:1) to afford 0.535 g (57%) of white crystalline methylol **41**: mp 99.5–100 °C (recrystallized from ethyl acetate–hexane; IR (film) 3310 (br), 2945, 1750, 1645, 1535, 1210, 1090, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 7.82 (2 H, m), 7.50 (3 H, m), 5.80 (3 H, m), 4.65 (2 H, d, J = 5.2 Hz), 4.60 (2 H, br), 1.70 (3 H, d, J = 6.6 Hz).

Methylol **41** (0.166 g, 0.666 mmol) was dissolved in 10 mL of methylene chloride, and pyridine (0.55 mL, 6.80 mmol) and acetic anhydride (0.65 mL, 6.88 mmol) were added dropwise with stirring. A catalytic amount of 4-(dimethylamino)pyridine was added, and the mixture was stirred at room temperature for 5 min. The mixture was poured into 30 mL of water and extracted four times with 25 mL of methylene chloride. The organic layer was washed successively with 20 mL of 5% HCl solution, 20 mL of saturated $NaHCO_3$ solution, and 20 mL of water and dried over anhydrous magnesium sulfate. Removal of the solvent in vacuo yielded 0.193 g (100%) of acetate **42** as a colorless oil, which was judged by TLC and ^1H NMR to be sufficiently pure for use in the next step: IR (film) 3350 (br), 2930, 1750, 1675, 1525, 1235, 1030, 970, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 7.90–7.20 (5 H, m), 6.59 (1 H, d, J = 9.0 Hz), 5.70 (2 H, m), 4.62 (2 H, d, J = 5.2 Hz), 2.12 (3 H, s), 1.68 (3 H, d, J = 4.6 Hz); mass spectrum, m/z (relative intensity) 231 (9), 192 (14), 150 (31), 105 (100), 77 (24), 43 (16).

Acetate **42** (0.057 g, 0.20 mmol) was dissolved in 5 mL of *o*-dichlorobenzene, and the solution was refluxed for 20 h. The solvent was removed in vacuo, and the residue was purified by preparative TLC (ethyl acetate–hexane, 1:1) to yield 0.029 g (64%) of dihydrooxazine **45** as a colorless oil: IR (film) 2980, 1785, 1650, 1320, 1285, 1175, 1130, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 8.03 (2 H, d, J = 8.3 Hz), 7.43 (3 H, m), 4.54 (1 H, d, J = 7.5 Hz), 4.51 (1 H, dd, J = 10.1, 6.1 Hz), 4.22 (1 H, dd, J = 10.1, 1.5 Hz), 3.98 (1 H, dq, J = 10.3, 6.2 Hz), 2.53 (1 H, dddd, J = 10.3, 7.5, 6.1, 1.5 Hz), 1.54 (3 H, d, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 173.67, 157.50, 132.57, 131.16, 127.99, 127.65, 69.13, 66.43, 54.60, 37.68, 18.96; mass spectrum, m/z (relative intensity) 231 (28), 105 (100), 77 (24), 28 (12); exact mass calcd for $C_{13}H_{13}NO_3$ 231.0911, found 231.0903.

Acknowledgment. We grateful to the National Science Foundation (CHE-81-00132) and the National Institutes of Health (CA-34303) for support of this research. SMW thanks the John Simon Guggenheim Memorial Foundation for a Fellowship (1983–1984).

Registry No. **1**, 2292-87-7; **2**, 83670-53-5; (±)-**5**, 93455-11-9; (±)-**6**, 93455-12-0; (±)-**7**, 93455-13-1; (±)-**7**·Na, 93455-14-2; (±)-**10**, 93455-15-3; (±)-**18**, 93455-16-4; (±)-**19**, 83670-59-1; **20**, 1509-35-9; **20** ethyl ester, 3082-86-8; (±)-**22**, 93455-17-5; (±)-**23**, 93455-18-6; **24**, 73-32-5; **24** ethyl ester, 921-74-4; **25a**, 93455-19-7; **25b**, 93455-20-0; **25c**, 93455-21-1; **26a**, 90449-00-6; **26b**, 93455-22-2; **26c**, 93455-23-3; **27a**, 93455-24-4; **27b**, 93455-25-5; **27c**, 93455-26-6; **28**, 93455-27-7; **29**, 93455-28-8; (±)-**30**, 93455-29-9; **31**, 93455-30-2; (±)-**32**, 93455-31-3; (±)-**33**, 93455-32-4; (±)-**34**, 93455-33-5; **37**, 93455-34-6; **38**, 28482-69-1; **39**, 81793-17-1; **40**, 93455-35-7; **41**, 93455-36-8; **42**, 93455-37-9; (±)-**45**, 93455-38-0; ethyl glyoxylate, 924-44-7; cyclohexene, 110-83-8; *trans*-2-butene, 624-64-6; *cis*-2-butene, 590-18-1; *N*-sulfinyl-*p*-toluenesulfonamide, 4104-47-6; benzamide, 55-21-0; (*E*)-3-hexen-1-ol, 928-97-2; (*E*)-2-buten-1-ol, 504-61-0; (*Z*)-3-hexen-1-ol, 928-96-1; bromoacetyl bromide, 598-21-0.

New Reaction of L-Ascorbic Acid: Unusual Molecular Complexes of the Product[1]

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2-Methyl-2,5-dimethoxy-2,5-dihydrofuran (**4**), a cyclic acetal of *cis*-3-acetylacrolein (**3**), reacts with L-ascorbic acid (**1**) in aqueous solution to give amorphous 2-(5-methyl-2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal (**6**) as the major product. The reaction mechanism most likely involves *cis*-3-acetylacrolein, i.e., 4-keto-*cis*-2-pentenal (**3**) as an intermediate. Hemiketal **6** was converted with succinic anhydride into a crystalline molecular complex **8a**. X-ray structure determination shows that **8a** is held together by strong hydrogen bonds between the succinic carbonyl oxygens and the C-3 hydroxyls of 2 mol of hemiketal **6**. Succinimide and *N*-methylsuccinimide also give very stable molecular complexes **8b** and **8c**, while maleic anhydride and *N*-phenylsuccinimide do not form crystalline adducts with **6**. The lactone **6** and its adducts show remarkable immunomodulation and an extremely low toxicity.

Introduction

Relatively little work has focused on the chemistry of Vitamin C since its practical syntheses[2a,b] were achieved. We described recently[3] a spontaneous reaction of L-ascorbic acid (**1**) with methylglyoxal. A mixture of isomeric ene diol acetals[3,4] formed by the loss of 1 mol of water in

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(1) Fodor, G.; Sussangkarn, K.; Arnold, R.; Karle, I.; George, C. "Abstracts of Papers", 186th National Meeting of the American Chemical Society, Washington, D.C., August 29, 1983; American Chemical Society: Washington, D.C., 1983; ORGN 75.

0022-3263/84/1949-5064$01.50/0

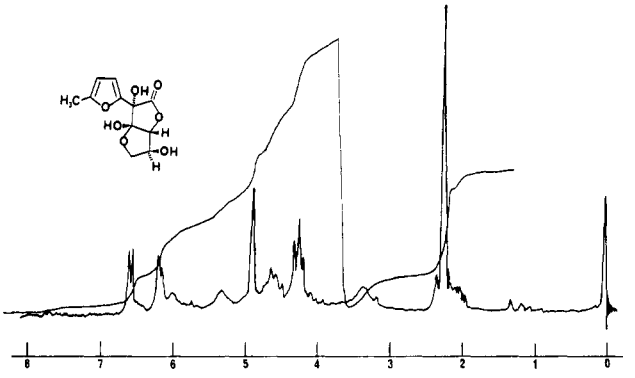
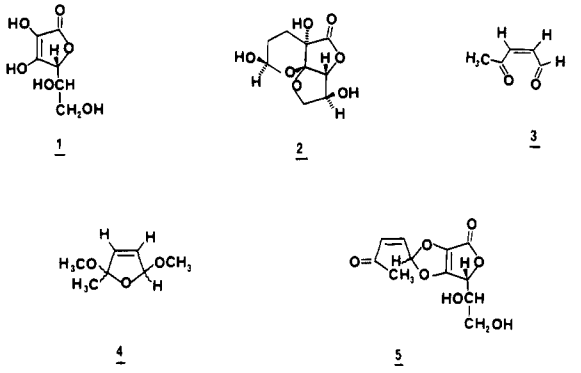


Figure 1. ^1H NMR spectrum of **6** in acetone-d_6.

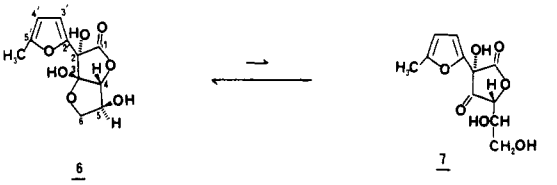
a reversible manner. In contrast, an unexpected Michael addition between **1** and acrolein in water gave a crystalline tricyclic derivative of 2-(3-oxopropyl)-3-keto-L-gulonolactone, the structure **2** of which has been established by X-ray crystallography.[5a,b]



Results

We found more recently that the vinylogue of methylglyoxal, 4-keto-*cis*-2-pentenal (**3**), in the form of its cyclic methyl acetal–methyl ketal **4**, reacts with a concentrated aqueous solution of **1** with the loss of 2 mol of methanol. The amorphous product analyzes for $C_{11}H_{12}O_7$, a molecular formula which would be consistent with the ene diol acetal structure **5**, i.e., a structure analogous to that of the products from methylglyoxal and **1**. The IR spectrum shows a lactone (1780 cm^{-1}), a carbonyl around 1700 cm^{-1}, and a C=C bond (1650 cm^{-1}) which can be reconciled with **5**. The ^1H NMR spectrum (Figure 1) clearly shows two olefinic protons, a doublet at δ 6.57 (J = 3.2 Hz) and an unresolved signal at δ 6.20. The singlet at δ 2.22 could be interpreted as a methyl group attached to a keto function. All other resonances can be attributed to the ascorbic acid moiety in the product. However, there is no evidence for a proton attached to an acetal carbon which should be present in ene diol acetal **5**. The ^{13}C NMR spectrum of the amorphous solid shows 12 major peaks. Two of those signals are close (106.5 and 107.0 ppm). They could be indicative of the acetal carbons in two diastereoisomeric forms of **5**. A keto carbonyl signal at 206.8 ppm was in most cases very weak or even escaped detection, in contrast to the lactone carbonyl at 173.0 ppm. Hence we had to consider structural alternatives.

A Michael-type addition of **1** to **3** could be ruled out, since that should have caused the disappearance of unsaturation and would not explain the loss of 1 mol of water. However, the knowledge gained by studying the Michael addition between **1** and acrolein[5a,b] eventually led to a different structural proposal. The alternative structure **6** is that of 2-(5-methyl-2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal. The ^{13}C chemical shifts of the carbons in the ascorbic acid derived moiety of **6** are in good agreement with values we have found[5b] for Michael adducts of **1**. That means the double bond in **1** most likely disappeared and, in a simultaneous or subsequent reaction, the "side chain" of ascorbic acid cyclized to form the 3,6-hemiketal. Two pairs of low-field ^{13}C resonances—107.0, 109.2 ppm and 148.5, 151.7 ppm—compare very well with respective values for 2,5-disubstituted furans found in the literature, and they can be assigned to C-3, C-4 and C-2, and C-5 of the furan ring in **6**, respectively. The furan structure is also supported by the chemical shifts and the small coupling constant (J = 3 Hz) of the olefinic protons in the ^1H NMR spectrum. The most significant difference between structures **5** and **6** is the position of the methyl carbon resonance: 2-methylfurans show ^{13}C signals around 13 ppm, while the methyl carbon adjacent to a keto carbonyl usually appears between 20 and 30 ppm. In our case the methyl carbon of **6** is found at 13.2 ppm. The significance of the signal at 206.8 ppm was questionable due to its weakness. It could be explained by assuming a tautomeric equilibrium between **6** and **7**. Another confusing fact was the positive iodoform test that seemed to indicate a $CH_3C{=}O$ group, consistent only with **5**.



The difficulty of a final structure determination lay in the amorphous state of our compound. Thus the preparation of a crystalline derivative was imperative, the more so since the compound showed strong immunomodulatory properties[4] and the reproducibility of those results depended on a completely pure specimen. Attempts to acylate the assumed free primary hydroxyl of **5** with *p*-nitrobenzoyl chloride in the presence of an organic base failed to give any crystalline product.

Fortunately, however, refluxing the amorphous compound with succinic anhydride in ethyl acetate leads to the separation of white crystals. Elemental analysis fits with an addition compound between 2 mol of the amorphous compound and 1 mol of succinic anhydride instead of the expected acid succinate of **5** or **7**. The IR spectrum indicates a lactone (1780 cm^{-1}), an anhydride carbonyl absorption (1820 cm^{-1}), but no keto carbonyl at 1700 cm^{-1}. The ^1H NMR spectrum (Figure 2) (supplementary material, see paragraph at the end of the paper) was superimposable with that of the starting material (Figure 1) except for an additional sharp singlet δ 3.10 for the succinic methylene protons. The ratio of methyl to methylene protons is 3:2 instead of 3:4 which is consistent with a 2:1 adduct. The ^{13}C NMR (Figure 3) (supplementary material) shows no ketone carbon but gave a sharp peak at 28.5 ppm for the succinic anhydride methylene carbons, oth-

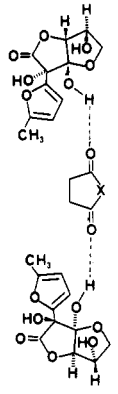
(2) (a) Haworth, R. N.; Hirst, E. L. *Helv. Chim. Acta* **1934**, *17*, 520. (b) Reichstein, T.; Grussner, A.; Oppenauer, R. *Ibid.* **1934**, *17*, 510–520.

(3) Fodor, G.; Mujumdar, R.; Butterick, J. In "Submolecular Biology and Cancer"; CIBA Foundation Symposium 67 (new series); Excerpta Medica: Amsterdam, 1979; pp 165–174.

(4) Arnold, R.; Fodor, G.; Mathelier, H.; Mohacsi, T.; Szent-Györgyi, A.; Veltri, R. W. In "Protective Agents in Cancer"; McBrien, D. C. H., Slater, T., Ed.; Academic Press: London, 1983; pp 192–219.

(5) (a) Fodor, G.; Butterick, J.; Mathelier, H.; Mohacsi, T. "Abstracts of Papers", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, April 12, 1982; American Chemical Society: Washington, D.C., 1982; ORGN 232. (b) Fodor, G.; Arnold, R.; Mohacsi, T.; Karle, I.; Flippen-Anderson, J. *Tetrahedron* **1983**, *39*, 2137–2145.

erwise it is superimposable with the spectrum of the amorphous solid. The anhydride carbonyl carbons are masked by the lactone resonance at 173.1 ppm. The mass spectrum of the complex (Figure 4) (supplementary material) shows m/e 256.2 as the highest peak which corresponds to component **6** of the adduct. The base peak m/e 109 can be assigned to the resonance-stabilized methylfuroyl cation. Succinic anhydride is known to produce fragment m/e 56 by loss of CO_2. This result indicates that the molecular complex dissociates into its components. Concerning NMR and mass spectra, Figures 2, 3, and 4, of compound **8a**, see paragraph at the end of the paper.



8a X = O
8b X = NH
8c X = NCH_3

Discussion

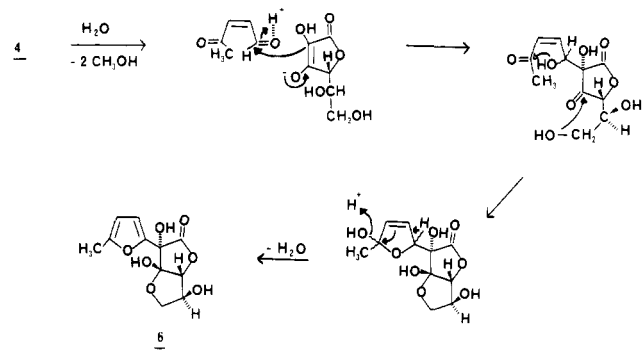
The X-ray study proves structure **6** of 2-(5-methyl-2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal which has the absolute configuration 2*S*,3*S*,4*R*,5*S*, and it ruled out the possibility of the ene diol acetal **5**. Since the C-3 hemiketal hydroxyl is involved in hydrogen bonding to succinic anhydride the tautomeric keto form **7** cannot be present in the crystalline state. In the early experiments the yield of molecular complex **8a** increased with the time of refluxing. This fact seemed to indicate that a tautomeric change may precede H bond formation. However, when the preparation of **6** was carried out from equimolar amounts of ascorbic acid and the acetal **4**, no delay was observed in the precipitation of **8a** in the next step.

We tried to find out the limitations of this kind of molecular complex formation. Succinimide reacts faster and gives a higher yield of a 2:1 molecular complex **8b** than succinic anhydride, probably because of the higher nucleophilicity of the carbonyl in the imide. The IR spectrum is distinguished from that of **6** solely by the appearance of the imide carbonyl at 1700 cm^{-1}.

The ^1H NMR spectra of **8b** and **6** are superimposable except for the additional methylene in **8b**. The ^{13}C NMR spectrum shows the succinimide carbonyl and the methylene in addition to the signals of **6**. The highest peak in the mass spectrum of the succinimide adduct is the M^+ peak of **6**. In addition, ionized succinimide appears at m/e 99 along with some other fragments of the imide. On the basis of these data the structure **8b** is proven without the need for X-ray structure determination. *N*-Methylsuccinimide reacts likewise, while *N*-phenylsuccinimide failed to give a crystalline adduct with **6**. The reason for this failure may be either electronic, i.e., decreased nucleophilicity of the carbonyl oxygen, or the inability of the *N*-phenyl ring to fit in the crystal lattice.

Preliminary experiments show that the cyclic acetal of maleic aldehyde, 2,5-dimethoxy-2,5-dihydrofuran, also

Scheme I



reacts with **1** to give a product, the NMR spectral data of which are consistent with the structure of 2-(2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal. However, monitoring the reaction by HPLC showed that the disappearance of ascorbic acid was still not complete after 4 days while the methyl homologue **4** consumed ascorbic acid within 1 h. This striking difference in reaction rates is hard to rationalize without further studies. Unfortunately, this furyl derivative, "nor-**6**", failed to give any crystalline adduct with succinic anhydride or succinimide.

In order to learn more about the mechanism of the new reaction *cis*-3-acetylacrolein (**3**) was prepared by 24 h hydrolysis of **4** with water[10] at 20 °C followed by extraction and distillation. The ^1H NMR spectrum in aprotic solvents proved that **3** was pure. Keto aldehyde **3** reacts with **1** in water at a somewhat higher rate than acetal **4** and with a comparable yield. In methanol, however, it reacts with **1** slowly over 11 days to give **6** in a 17% yield.

The mechanism of formation of **6** apparently involves a stereospecific nucleophilic attack by C-2 of ascorbic acid (**1**) upon the aldehyde carbon of **3**. Alternatively the C-5 of the dihydrofuran **4** could undergo an S_N2-type nucleophilic reaction, catalyzed by the electrophilic attack of the ascorbic acid proton at one of the methoxyl groups of **4**. This alternate pathway is ruled out by the fact that acetal **4** does not react at all with ascorbic acid in pure methanol.

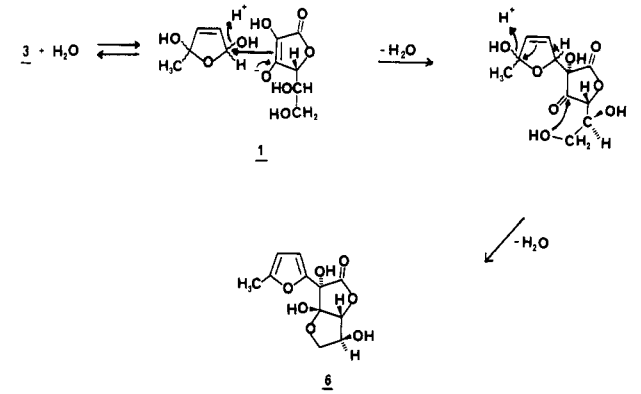
Therefore one has to assume the intermediacy of *cis*-3-acetylacrolein (**3**) which, indeed, reacts both in aqueous and methanolic solution with **1**. A proposed mechanism is depicted in Scheme I. However, the ^1H NMR spectrum of **3** in D_2O shows a gradual upfield shift of the methyl signal from δ 2.4 to 1.5, which then appears as a doublet, and a slow decrease of the doublet for the aldehyde proton at δ 10.2. In methanol-d_3 a similar change occurs. These shifts can only be interpreted in terms of hydration or hemiacetal–hemiketal formation involving the ketone and the aldehyde groups, possibly simultaneously. Therefore, the reactive species might be the cyclic hemiacetal, 2-methyl-2,5-dihydroxy-2,5-dihydrofuran, that would react with the ascorbate carbanion according to a push–pull mechanism (Scheme II). Further studies are needed to monitor and clarify this reaction.

In contrast to maleic aldehyde, fumaric aldehyde and ascorbic acid give a crystalline product, $C_{10}H_{12}O_8$, that shows only a lactone carbonyl in the IR and the ^{13}C NMR spectrum but no olefinic protons or carbons are indicated. Therefore, an addition to rather than condensation with **1** had taken place. X-ray crystallographic work is underway to determine the structure of the fumaric aldehyde derivative.

Conclusion

The stereospecificity of the attack of the ascorbate carbanion upon **3** or **4** is remarkable. It is also significant

Scheme II



that maleic and fumaric aldehyde react via individual mechanisms and give different products in a stereospecific way. The best explanation for this is to assume that maleic aldehyde and 3-acetylacrolein are present in a preformed furan state (Scheme II) which gives preference to the formation of a 2-(2-furyl)-3-keto-L-gulonolactone derivative, while fumaric aldehyde being unable to assume a similar geometry undergoes a concomitant Michael-type reaction on either of the β-carbon atoms. The selectivity of molecular complex formation with succinic anhydride and succinimides is likewise a fascinating question.

Experimental Section

Melting points were determined on a Melt-Temp apparatus and are uncorrected. IR spectra were recorded on the Beckman IR-8 and Beckman IR 10 spectrophotometers. ^{13}C NMR spectra were taken on a Varian CFT-20 spectrometer at 20 MHz. ^{1}H NMR spectra were recorded on a Varian EM-360 (60 MHz) spectrometer. A Varian Model 5000 liquid chromatograph equipped with a reverse-phase MCH-10 column was used for HPLC works. Mass spectra were obtained on a Finnigan 4021 mass spectrometer with an INCOS data system. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Optical rotations were measured on a Perkin-Elmer Model 141 Polarimeter. 2-Methyl-2,5-dimethoxy-2,5-dihydrofuran was prepared as described by Clauson-Kaas and Lumborg.[11] L-Ascorbic acid U.S.P. (Mallinckrodt) and succinimide (Aldrich) were used as purchased. Succinic anhydride (Aldrich) was recrystallized from acetic anhydride before use.

2-(5-Methyl-2-furyl)-3-keto-L-gulonolactone 3,6-Hemiketal (6). L-Ascorbic acid (1) (150.0 g, 0.852 mol) was dissolved in 750 mL of distilled water that had been purged with nitrogen for 1.5 h. Freshly distilled 2-methyl-2,5-dimethoxy-2,5-dihydrofuran (4) (124.0 g, 0.86 mol) was added dropwise with stirring over a period of 1.5 h. After the addition was completed, the reaction mixture was left stirring under a nitrogen atmosphere for 24 h. The yellow solution was frozen and freeze-dried on a Virtis Freezemobile at 10–50 mtorr for at least 7 days to give crude **6** (228.1 g). The yellow crude **6** (100.0 g) was purified by shaking its ethyl acetate (500 mL) solution with neutral decolorizing carbon (Norit) (6 × 50.0 g) until a colorless solution resulted. After the removal of Norit by filtration, the solvent was evaporated to dryness to give powdered, amorphous **6** (54.8 g), sinters at 45–47 °C: IR (KBr) 3600–3100 (s, broad, OH), 1785 (s, lactone CO), 1700 (m, CO), 1610 and 1540 (w, furan C=C) cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.57 (d, 1 H), 6.20 (m, 1 H), 4.87 (s, 1 H), 4.63 (m, 1 H), 4.25 (m, 2 H), 2.22 (s, 3 H); ^{13}C NMR (Me_2SO-d_6) 206.8, 173.0, 151.7, 148.5, 109.2, 107.0, 106.5, 87.6, 77.6, 74.7, 73.7, 13.2 ppm; mass spectrum, *m/e* 256.1, 239.1, 155.0, 138.0, 109.0 (base peak), 85.0. Anal. ($C_{11}H_{12}O_7$) C, 51.70; H, 4.80; O, 43.57. The crude product **6** can be converted into its molecular complexes without purification.

Preparation of the Molecular Complex 8a from 6 and Succinic Anhydride. Crude **6** (128.0 g, 0.5 mol) was dissolved in 290 mL of ethyl acetate. Recrystallized succinic anhydride (50.0 g, 0.5 mol) was added, and the mixture was stirred under nitrogen and heated to reflux for 16 h. The homogeneous reaction mixture was allowed to cool to room temperature with stirring whereupon a white solid precipitated. After being kept in an ice bath for 2 h the solid material was filtered, washed with 100 mL cold ethyl acetate, and dried under vacuum to give 78.2 g of crude **8a**. Chloroform (390 mL) was added to crude **8a** in order to remove unreacted succinic anhydride. The suspension was stirred at room temperature under nitrogen for 1 h. The solid was filtered by suction and dried under vacuum to give purified **8a** (69.0 g) which was recrystallized from chloroform–ethyl acetate (80:20) to give long white needles (43.3 g, 33.8%): mp 134–134 °C; $[\alpha]^{22}$ +56.1° (*c* 1.02, ethyl acetate); IR (KBr) 3400 (s, broad, OH), 1790 (s, lactone CO), 1850 and 1770 (s, anhydride CO), 1610 and 1560 (w, furan C=C) cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.53 (d, 1 H), 6.17 (m, 1 H), 5.27 (broad, HDO), 4.83 (s, 1 H), 4.60 (m, 1 H), 4.23 (m, 2 H), 3.10 (s, 2 H), 2.27 (s, 3 H); ^{13}C NMR (Me_2SO-d_6) 173.10, 151.98, 148.14, 109.35, 107.00, 106.57, 87.48, 77.58, 74.78, 73.48, 28.50, 13.13 ppm (for assignments see Figure 3); mass spectrum, *m/e* 256, 239, 228, 155, 138, 121, 110, 109, 101, 95, 85, 71, 56 (see Figure 4). Anal. ($C_{26}H_{28}O_{17}$) C, 51.01; H, 4.72; O, 44.40.

X-ray Crystallography. The compound 2-(5-methyl-2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal (**6**) co-crystallizes with succinic anhydride in a 2:1 ratio in the tetragonal space group $P4_2$ in which the succinic anhydride occupies a special position within the cell. A crystallographic 2-fold axis passes through the anhydride ring, i.e., through the ring oxygen and the midpoint of the C3″–C3″ bond. The unit cell, $a = b = 14.784$ (2) Å, $c = 6.235$ (1) Å and $Z = 4$, contains four molecules of **6** and two molecules of succinic anhydride with a calculated density, $d_x = 1.493$ gm/cm. The 997 independent reflections were collected on a Nicolet P3F automatic X-ray diffractometer with Mo Kα radiation with a graphite incident beam monochrometer. The structure was solved by direct methods with the use of the program[6] MULTAN 80 and the atom positions and anisotropic thermal factors for the C and O atoms were refined by full-matrix least-squares methods with program[7] ORFLXS3. The hydrogen positions, located by difference maps calculated after several cycles of refinement on only the C and O parameters, were refined while the thermal parameters for the hydrogens were fixed at the values of the atoms to which they were bonded. In the least-squares refinement the function minimized was $\sum\omega(|F_O| - |F_C|)^2$, where the weights, $\omega = \sigma^{-2}(|F_O|)$, are derived from the counting statistics of the observed data and a term for estimating random errors[8] (0.02 in this work). The final *R* factors are $R = 3.7\%$ and $\omega R = 3.2\%$ for the 872 reflections where $|F_O| > 3\sigma(|F_O|)$ and $R = 5.0\%$ for all the 997 independent reflections.

$$R = \frac{\sum||F_O| - |F_C||}{\sum F_O}$$

$$\omega R = \left\{\frac{\sum\omega(|F_O| - |F_C|)^2}{\sum\omega(F_O)^2}\right\}^{1/2}$$

Table I lists the fractional coordinates for the atoms as well as the equivalent isotropic thermal parameters for the C and O atoms. Bond distances and angles for the molecular complex **8a** are given in Figure 6. The anisotropic thermal parameters and a comparison of the observed and calculated structure factors and phases are available from the authors.

The position of the two molecules with respect to one another is depicted in the diagram Figure 5, drawn by the computer program[9] ORTEP using experimentally determined coordinates. Shown is one molecule of each compound. The absolute configuration of compound **6** is based on the chirality of ascorbic acid

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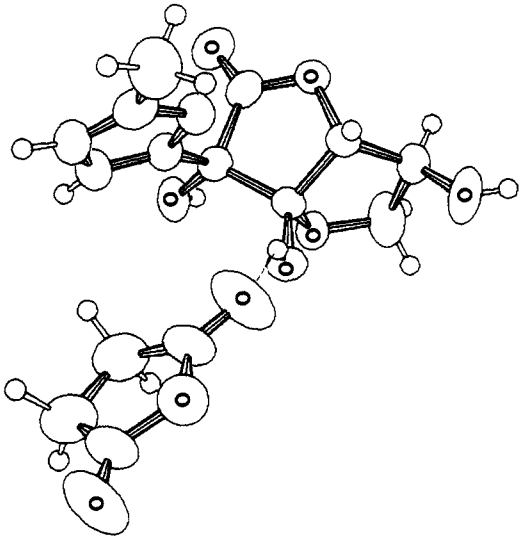

Figure 5. Diagram of **8a** as determined by X-ray diffraction showing the succinic anhydride and one molecule of **6**. The second molecule of **6** is hydrogen bonded to the other carbonyl oxygen of succinic anhydride.

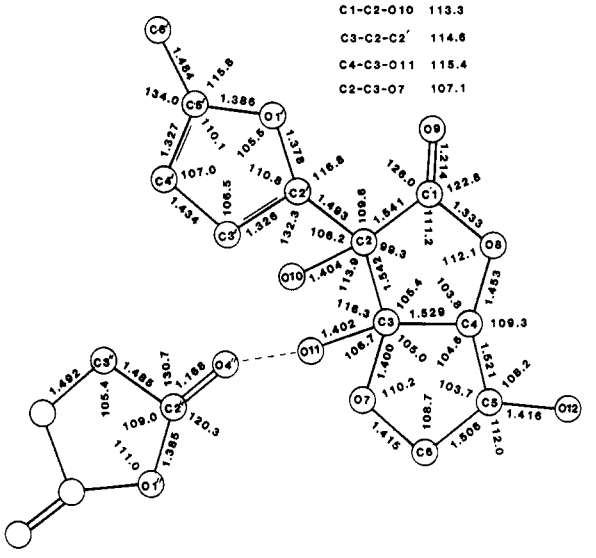

Figure 6. Bond lengths (esd's 0.006 Å) and bond angles (esd's 0.5°) in **8a**.

(1) as referenced to the four chiral centers of **6**. The two fused five-membered rings are twisted about their common bond such that the torsion angles formed by O8–C4–C3–C2 and O7–C3–C4–C5 are 25.8° and 27.5°, respectively, while two torsion angles, O8–C4–C3–O7 and C2–C3–C4–C5, are 87.0° and −140.4°, respectively. The least-squares planes through each of the two rings form a dihedral angle of 108.5°; here the average deviation of atoms from their respective planes is 0.1 Å. The methyl furyl ring is planar having an average deviation of 0.001 Å from the least-squares plane and a dihedral angle of 90.5° with the least-squares plane through the lactone ring. The succinic anhydride ring is also planar with the average deviation from the plane of 0.004 Å.

Crystal packing of the two molecules is influenced by hydrogen bonding. The carboxyl oxygens of succinic anhydride are hydrogen bonded to hydroxyl H11–O11 of **6** with an H11–O4″ distance of 2.05 Å, an O11–O4″ distance of 2.78 Å, and an O11–H11–O4″ angle of 137.4°. Hydrogen bonding also occurs with O10 as a donor to O12, and O12 as a donor to O11. The respective H–O distance, O–O distance, and angle are 2.02 Å, 2.74 Å, and 162.1° and 2.08 Å, 2.85 Å, and 162.2°. An additional intermolecular approach less than the van der Waals separation is the O9–C2″ distance of 3.19 Å.

Preparation of the Molecular Complex 8b from 6 and Succinimide. To a solution of crude **6** (119.65 g, 0.467 mol) in ethyl acetate was added succinimide (23.3 g, 0.235 mol), and the mixture was stirred under nitrogen. After a few minutes a white crystalline precipitate formed. The solid redissolved on heating. The solution was allowed to cool slowly to room temperature and then kept in an ice bath for 2 h. The crystals were filtered off, washed with cold ethyl acetate, and dried under vacuum to give 67.4 g of complex **8b** which was recrystallized from ethyl acetate–chloroform (60:40) to give long white needles (52.2 g, 43.6%): mp 132–133 °C; $[\alpha]^{22}$ +55.3° (*c* 0.85, ethyl acetate); IR(KBr) 3400 (s, broad, OH), 1790 (s, lactone CO), 1700 (s, imide CO), 1650 and 1560 (w, furan C=C) cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.60 (d, 1 H), 6.20 (m, 1 H), 5.37 (broad, HDO), 4.90 (s, 1 H), 4.67 (m, 1 H), 4.30 (m, 2 H), 2.73 (s, 2 H), 2.27 (s, 3 H); ^{13}C NMR (Me_2SO-d_6) 179.39, 173.04, 151.69, 148.39, 109.14, 106.98, 106.45, 87.53, 77.58, 74.61, 73.53, 29.42, 13.17 ppm; mass spectrum, *m/e* 256, 239, 228, 155, 138, 121, 110, 109, 99, 95, 85, 71, 56. Anal. ($C_{26}H_{29}NO_{16}$) C, 51.17; H, 4.93; O, 41.81; N, 2.21.

***N*-Methylsuccinimide.** The general—but not detailed—method for preparing *N*-alkylsuccinimides[12] from potassium succinimide and an alkyl iodide was applied here. When 10 g of potassium succinimide in 50 mL of absolute alcohol, 10 mL of chloroform, and 10 mL of methyl iodide were used, followed by refluxing for 30 min, 5.1 g (61.3%) of pure *N*-methylsuccinimide, mp 64–66 °C, were obtained: ^{1}H NMR ($CDCl_3$) δ 3.03 (s, 3 H), 2.75 (s, 4 H).

Preparation of the Molecular Complex 8c from 6 and *N*-Methylsuccinimide. The method described for **8b** was followed by using crude **6** (10.24 g, 0.04 mol) and *N*-methylsuccinimide (2.5 g, 0.02 mol) in 20 mL of ethyl acetate. The crude complex **8c** was recrystallized from ethyl acetate–chloroform (50:50) to give 3.21 g (31.3%) of pure **8c**: mp 105–106 °C; $[\alpha]^{220}$ +54.4° (*c* 1.03, ethyl acetate); IR (KBr) 3360 (s, broad, OH), 1790 (s, lactone CO), 1680 (s, imide CO), 1620 and 1560 (w, furan C=C) cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.57 (d, 1 H), 6.17 (m, 1 H), 4.85 (s, 1 H), 4.60 (m, 1 H), 4.27 (m, 2 H), 3.20 (broad, HDO), 2.90 (s, 1.5 H), 2.70 (s, 2 H), 2.25 (s, 3 H); ^{13}C NMR (Me_2SO-d_6) 177.73, 172.93, 151.60, 148.34, 109.06, 106.85, 106.38, 87.50, 77.48, 74.54, 73.50, 27.99, 24.13, 13.17 ppm; mass spectrum, *m/e* 256, 239, 228, 155, 113, 111, 110, 109, 95, 85, 71, 56. Anal. ($C_{27}H_{31}NO_{16}$) C, 51.98; H, 5.12; N, 2.08; O, 40.63.

***cis*-3-Acetylacrolein (3).** A modified version of the method by Hirsch and Szur[10] was used. 2-Methyl-2,5-dimethoxy-2,5-dihydrofuran (**4**) (100.0 g, 0.694 mol) was suspended in 350 mL of distilled water. After stirring for 30 min the mixture became homogeneous. The yellow solution was stirred under nitrogen for 24 h at room temperature and subsequently extracted with methylene chloride (5 × 200 mL). The extracts were combined and dried over anhydrous $MgSO_4$. The solvents were removed on a rotary evaporator and the residue was subjected to fractional distillation under reduced pressure. The forerun (72–78 °C (20 mm)) consisted mainly of **4**. The fraction boiling at 78–80 °C (18 mm) was collected and shown to be pure *cis*-3-acetylacrolein (36.7 g, 54.0%). Hirsch and Szur reported[10] bp 79 °C (20 mm) and 43.7% yield. IR (Neat) 1680 (s, CO), 1600 (s, C=C), 1360 (s, CH_3) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.23 (d, 1 H), 7.13 (d, 1 H), 6.28 (dd, 1 H), 2.37 (s, 3 H). Anal. ($C_5H_6O_2$) C, 60.94; H, 6.27.

Formation of 6 from 1 and *cis*-3-Acetylacrolein (3). L-Ascorbic acid (**1**) (27.6 g, 0.157 mol) was dissolved in 165 mL of degassed water. *cis*-3-Acetylacrolein (19.2 g, 0.196 mol) was added dropwise with stirring. HPLC analysis showed the complete disappearance of L-ascorbic acid 35 min after the addition. The reaction mixture was left stirring under nitrogen for 4 h, then frozen, and freeze-dried. A yellow amorphous foam was obtained and converted, as described above, into the molecular complex **8b** (24.58 g, 56.3%). All physical and spectral properties confirmed the identity of **8b**.

Formation of 6 from 1 and 3 in Absolute Methanol. L-Ascorbic acid **1** (7.22 g, 0.041 mol) reacted with *cis*-3-acetylacrolein (4.47 g, 0.045 mol) in absolute methanol (250 mL) to give after 11 days an amorphous powder (1.79 g, 17.1%), the spectral data of which confirmed its identity with compound **6**.

Analogous reaction between **1** and **4** under identical conditions did not produce **6**.

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Formation of Nor-6 from Ascorbic Acid and the Cyclic Acetal of Maleic Aldehyde. L-Ascorbic acid (1) (35.2 g, 0.20 mol) was dissolved in 112 mL of distilled water that had been purged with nitrogen for 1 h. Freshly distilled 2,5-dimethoxy-2,5-dihydrofuran (Aldrich Chemical Co.) (39.0 g, 0.30 mol) was added at once and the reaction mixture was left stirring under a nitrogen atmosphere for 10 days. The yellow-colored solution was frozen and freeze-dried on a Virtis Freezemobile at 10–50 mtorr for 7 days to give a crude product (50.4 g). The yellow solid was purified with Norit the same way as described for **6**. The colorless, amorphous 2-(2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal (17.6 g, 36.4%) was obtained: ^{1}H NMR (acetone-d_6) δ 7.60 (d, 1 H), δ 6.65 (m, 1 H), δ 6.53 (m, 1 H), δ 4.87 (s, 1 H), δ 4.62 (m, 1 H), δ 4.23 (m, 2 H); ^{13}C NMR (Me_2SO-d_6) 172.9, 150.2, 143.0, 110.5, 108.4, 106.9, 87.6, 77.6, 74.6, 73.5.

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Registry No. 1, 50-81-7; 3, 34218-22-9; 4, 22414-24-0; 6, 93135-89-8; nor-6, 93135-92-3; 8a, 93184-28-2; 8b, 93135-90-1; 8c, 93135-91-2; succinic anhydride, 108-30-5; succinimide, 123-56-8; *N*-methylsuccinimide, 1121-07-9; potassium succinimide, 50433-20-0; 2,5-dimethyl-2,5-dihydrofuran, 332-77-4.

Supplementary Material Available: Full NMR and mass spectral data of complex **8a** (Figures 2, 3, 4) and fractional coordinates for **8a** (Tables 1 and 2) (5 pages). Ordering information is given on any current masthead page.

Conversion of 2,3-Dihydropyrrolo[2,3-*b*]indoles to 2-(Alkylthio)-L-tryptophans

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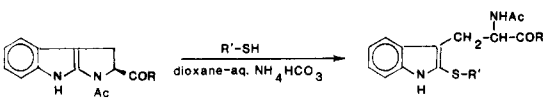
2,3-Dihydropyrrolo[2,3-*b*]indoles, easily derived from tryptophan derivatives by oxidation, are efficiently converted (48–85%) to 2-(alkylthio)tryptophan derivatives with thiols such as mercaptoethanol, ethanethiol, cysteine, or glutathione in ammonium bicarbonate buffered dioxane or dimethylformamide at 55 °C.

We have reported a novel conversion of *N*-acetyl-L-tryptophan esters, *N*-acetyl-L-tryptophanamide, and *N*-acetyltryptamine to the corresponding tricyclic 2,3-dihydropyrrolo[2,3-*b*]indoles by a one-step oxidation with aqueous *N*-bromosuccinimide at pH 9 or a preferable procedure using *tert*-butyl hypochlorite in triethylamine-buffered methylene chloride.[1,2] The tetracyclic system present in the sporidesmins, metabolites of *Pithomyces chartarum*,[3-5] was synthesized by *tert*-butyl hypochlorite oxidation of *N*-methyl-L-alanyl-L-tryptophan diketopiperazide.[2]

That these pyrroloindoles, themselves, can serve as useful intermediates is illustrated by their efficient conversion to 2-hydroxytryptophans by using sealed-tube hydrolysis with HCl. Crystalline 2-hydroxytryptophan, heretofore troublesome to prepare, is obtained by what is probably the most practical route at present.[6] This reaction, as well as the chlorination or acetoxylation of the 3a-position of 2,3-dihydropyrrolo[2,3-*b*]indoles[2] illustrate their use as reactive intermediates in the introduction of functional groups at the 2- or 3-position of the indole ring. In this paper, we extend the application of 2,3-dihydropyrrolo[2,3-*b*]indoles to the synthesis of 2-(alkylthio)-L-tryptophans.

Scheme I



1	R = NH_2		
2	R = OCH_3		
3	R = NH_2	R' = Et	
4	R = NH_2	R' = CH_2CH_2OH	
5	R = NH_2	R' = CH_2-CH(NH_2)CO_2H	
6	R = NH_2	R' = CH_2-CH(NH-γ-Glu)CO-Gly	
7	R = OCH_3	R' = Et	
8	R = OCH_3	R' = CH_2CH_2OH	
9	R = OCH_3	R' = $CH_2CH(NH_2)CO_2H$	
10	R = OCH_3	R' = CH_2CH(NH-γ-Glu)CO-Gly	

Results

We find that exposure of the pyrroloindoles **1** or **2** derived from *N*-acetyl-L-tryptophanamide or from *N*-acetyl-L-tryptophan methyl ester, respectively, with 2.5–10-fold molar excesses of the thiols, ethanethiol, 2-mercaptoethanol, L-cysteine, or glutathione, in dioxane or dimethylformamide containing ammonium bicarbonate at 55 °C gives 2-(alkylthio)tryptophans. The reaction must be conducted under weakly basic conditions, since under acidic conditions (pH <6), pyrroloindoles undergo spon-

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