rapidly to resemble those characteristic of the disulfide, indicate that 2-nitro-5-mercaptobenzoic acid is exceedingly sensitive to aerial oxidation.

Since it was not practical to prepare a sample of pure 2-nitro-5-mercaptobenzoic acid, its molar absorptivity was calculated from the absorbance of a solution of the disulfide in aqueous phosphate buffer to which sufficient aqueous solution of sodium thioglycolate had been added to produce maximal absorbance (Table II). A solution of exactly the same

TABLE II

ABSORPTION SPECTRAL CONSTANTS FOR CERTAIN AROMATIC DISULFIDES AND THE CORRESPONDING THIOLS

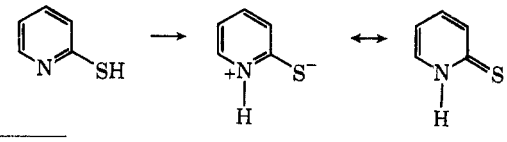
Compd	Registry no.	λ_{max}, nm	a_m
2,2′-Dinitro-5,5′-dithiodibenzoate[a]	552-24-9	325	17,500
		748	2,800
2-Nitro-5-mercaptobenzoate[b]	18430-02-9	412	13,600
		825	12,600
2,2′-Dithiodipyridine[c]	2127-03-9	233	13,900
		281	9,700
2-Mercaptopyridine[c]	2637-34-5	238	6,200
		343	8,700
		740	550
4,4′-Dithiodipyridine[d]	2645-22-9	247	16,100
4-Mercaptopyridine[d]	4556-23-4	230	9,600
		324	19,800
2,2′-Dithiodipyrimidine[c]	15718-46-4	237	19,000
2-Mercaptopyrimidine[c]	1450-85-7	278	21,000
		346	2,600
		780	795

[a] In aqueous phosphate buffer at pH 7.0. [b] The above disulfide solution to which sufficient sodium thioglycolate had been added to give maximal absorbance. [c] 0.1 N H_2SO_4. [d] Phosphate buffer at pH 7.2.

disulfide concentration, but 0.1 N in NaOH, gave exactly 0.75 of the absorbance of the previous solution, completely in agreement with reaction 3 and the observation of Donovan.[4]

The pK_a for the sulfhydryl group at 25°, determined spectrophotometrically, was found to be 4.75. Harrap[9] has reported a value of 4.8 ± 0.1 at 20°. From the recorded values[10] for 4-nitrothiophenol (4.77 at 30° in 40% aqueous ethanol) and for 3-mercaptobenzoic acid (6.15 at 28° in water) it is clear, as was expected, that the nitro group increases the acidity of thiophenol considerably more than does the carboxyl group.

An exactly parallel situation is presented by 2,2′- and 4,4′-dithiodipyridine and their nitro and carboxy derivatives, all of which have been recommended by Grassetti and Murray[7,11] as alternatives to Ellman's reagent for the determination of sulfhydryl. Albert and Barlin[12] have shown that 2- and 4-mercaptopyridine are uncommonly acidic thiols, by reason of the resonance



stabilization of the highly favored tautomer (pK_a values of −1.07 and +1.43, respectively). One would expect that the corresponding disulfides would be at least as susceptible to alkaline cleavage as Ellman's reagent, and such has proved to be the case (see Table III).

TABLE III

DECOMPOSITION OF SEVERAL HETEROCYCLIC DISULFIDES IN AQUEOUS SOLUTION AT 25° AS A FUNCTION OF pH

Compd	pH	Half-life, min
2,2′-Dithiodipyridine[a]	11.20	12
	10.60	58
	10.32	200
	9.92	>300
4,4′-Dithiodipyridine[b]	11.32	13
	10.52	67
	10.43	97
	9.83	>300
2,2′-Dithiodipyrimidine[c]	11.40	4
	10.40	40
	9.92	133
	9.55	>240

Decomposition followed by measurement of increase of absorbance at [a] 740 nm, [b] 324 nm, [c] 780 nm.

Experimental Section

Materials.—2,2′-Dinitro-5,5′-dithiodibenzoic acid was purchased both from Calbiochem, Los Angeles, Calif., and Aldrich Chemicals, Milwaukee, Wis. 2- and 4-Mercaptopyridine were purchased from Aldrich Chemical Co. 2-mercaptopyrimidine was obtained from Research Organic/Inorganic Chemicals, Sun City, Calif. Thioglycolic acid was a gift from Evans Chemetics, New York City. The disulfides were prepared by oxidizing aqueous solutions of the thiols with potassium triiodide: 2,2′-dithiodipyridine melted at 56–58° (lit. 57–58°); 4,4′-dithiodipyridine melted at 74–76° (lit. 74°); 2,2′-dithiodipyrimidine melted at 134–137° (lit. 139–140°).

Methods.—All melting points are uncorrected. Absorbance measurements given in Figure 1 were obtained with a Bausch & Lomb spectronic 20. Absorbance measurements required for determination of λ_{max} values, calculation of a_m values, and determination of pK_a values were obtained with a Beckman DB-G recording spectrophotometer.

Registry No.—2,2′-Dinitro-5,5′-dithiodibenzoic acid, 69-78-3.

Acknowledgment.—Grateful acknowledgment is made to National Institutes of Health (AM-13109) and to the National Science Foundation Undergraduate Research Participation Program.

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Synthesis of β-Substituted Pyrroles *via* 1-(Pyrrol-2-ylmethylene)pyrrolidinium Salts

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We wished to prepare 3-isoprenoid pyrroles for screening as arthropod antimaturants.[1] Such materials would be pyrrolic analogs of perillen and dendrolasin, substances isolated from the mandibular glands of an

(1) C. M. Williams, International Symposium on New Perspectives on the Control of Injurious Insects, Rome, Italy, Sept 16–18, 1968.

ant *Lasius (Dendrolasius) fuliginosus* (Latreille).[2] Dendrolasin has been reported to have juvenile hormone activity,[3] and it has been hypothesized that it acts as a defense substance.[4]

One method of preparing 3-substituted pyrroles involves synthesizing pyrroles substituted in the 2 position with a removable electron-withdrawing group. Electrophilic attack upon such a compound occurs more readily at the 4 position than at the normally more reactive 5 position. In this connection, conversion of 2-pyrrolecarboxaldehyde to a ternary iminium salt[5] seemed a useful way to protect the aldehyde group and to enhance its meta-directing influence in electrophilic substitution reactions. The free aldehyde could then be regenerated from the product salt and be removed by oxidation and decarboxylation to give a 3-substituted pyrrole.

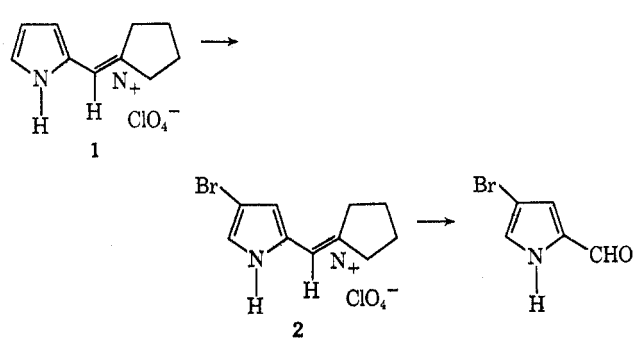
Salt **1** was obtained quantitatively by heating 2-pyrrolecarboxaldehyde with 1 equiv of pyrrolidinium perchlorate in benzene and removing the water azeotropically. The salt was brominated in ethylene dichloride and the product could then be isolated for characterization or converted to a bromoaldehyde by treatment of the crude salt with aqueous sodium bicarbonate. The bromination of 2-pyrrolecarboxaldehyde produces primarily 4-bromo-2-pyrrolecarboxaldehyde and minor amounts of the 5 isomer and the 4,5-dibromo compound.[6] A comparison of the relative quantities of these by-products (see Table I) reveals

TABLE I
PRODUCT DISTRIBUTION IN MOLE PER CENT

		Products		
Starting material	T, °C	4-Br	5-Br	4,5-diBr
2-Pyrrolecarboxaldehyde[a]	28	83.5	14.5	2
2-Pyrrolecarboxaldehyde[a]	0	97	3	
1[b]	26–28	96.5	3.5	
1[b]	0–5	99.5	0.5	

[a] Reference 6. [b] This work.

that bromination of the iminium salt derivative is considerably more selective than bromination of the free aldehyde. Also, the yield of the brominated aldehyde from the salt (~90%) is greater than the yield which we could obtain from the free aldehyde (~55%).



The directive ability of several other α-substituted electron-withdrawing groups has been investigated. The 2-alkoxycarbonyl group was only moderately meta directing for substitution reactions on pyrrole rings;[7] the 2-formyl and 2-cyano groups were more meta selective, but the transformations required for removal of these groups from the pyrrole ring did not produce high yields.[7a,b] Also, acetylation of 2-pyrrolecarboxaldehyde gave low yields attended by considerable decomposition.[7b] Only the 2-thiolcarboxylate group appeared useful for the elaboration of 3-substituted pyrroles by the "2-meta group" approach.[8]

Acetylation of **1** followed by hydrolysis provided 4-acetyl-2-pyrrolecarboxyaldehyde in 98–98.5% purity (glpc) and 77% yield. The iminium group, therefore, provides considerable selectivity for meta substitution and also gives greater yields of 4-substituted 2-pyrrolecarboxaldehydes. In addition, 4-acetyl-2-pyrrolecarboxylic acid was obtained from the aldehyde in 86% yield compared with a reported 38%,[7b] by using a continuous extraction technique for product isolation. Therefore, this approach appears to have some utility for the preparation of 3-substituted pyrroles. The chemistry of 1-(pyrrol-2-ylmethylene)pyrrolidinium salts is being further investigated.

Experimental Section

Infrared spectra were determined on both Perkin-Elmer Model 137 and 521 infrared spectrophotometers. Nmr spectra were obtained with a Varian T-60 instrument, and chemical shifts are reported in ppm from TMS. Glpc data were obtained with an Aerograph Model A-700 instrument and an SE-30 column (5% on acid-washed Chromosorb W, 10 ft × 0.125 in.) at 180–200°. Elemental analyses were carried out by Galbraith Laboratories Inc., Knoxville, Tenn. The mention of a proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

1-(Pyrrol-2-ylmethylene)pyrrolidinium Perchlorate (1).—Pyrrolidine (9.95 g) and 19.8 g of 70–72% $HClO_4$ in 50 ml each of C_6H_6 and EtOAc was heated under reflux with a Dean-Stark trap until H_2O was no longer expelled from the reaction mixture. Pyrrole-2-carboxaldehyde[9] (13.45 g) was added, and the resulting mixture was again heated under reflux to remove water (~0.5 hr). The solvent was evaporated, and the oily product was crystallized under Et_2O. The solid was filtered and air-dried to give 34.9 g (100%). Recrystallization from CH_3CN–Et_2O gave yellow needles: mp 101–102.5°; ir (mull) 3375 b, 1656 (C=N^+<);[1] nmr (DMSO-d_6) 2.1–2.5 (m, 4, β CH_2's), 3.8–4.4 (m, 4, α CH_2's), 6.67 (q, 1, $J_{3,4}$ = 4.1, $J_{4,5}$ = 2.5 Hz, 4 H), 7.37 (d, 1, J = 4.1 Hz, 3 H), 7.77 (bs, 1, 5 H), 8.78 (bs, 1, ArCH=N^+<).

Anal. Calcd for $C_9H_{13}ClN_2O_4$: C, 43.47; H, 5.27; Cl, 14.26; N, 11.27. Found: C, 43.51; H, 5.29; Cl, 14.33; N, 11.29.

1-[(4-Bromopyrrol-2-yl)methylene]pyrrolidinium Perchlorate (2) and 4-Bromo-2-pyrrolecarboxaldehyde.—Crude **1** (1.25 g) was dissolved in 25 ml of CH_2ClCH_2Cl, and 0.80 g of bromine dissolved in 10 ml of CH_2ClCH_2Cl was added dropwise to this solution (T 26–28°). After 1 hr at 28°, the mixture was concentrated to give 1.62 g (98.7%) of crude **2**. Recrystallization from CH_3CN–Et_2O gave mp 125–127.5°: ir (mull) 3340 b, 1652 (C=N^+<); nmr (DMSO-d_6) 2.1–2.5 (m, 4, β CH_2's), 3.8–4.4 (m, 4, α CH_2's), 7.52 (s, 1, 3 H), 7.92 (s, 1, 5 H), 8.65 (bs, 1, ArCH=N^+<).

Anal. Calcd for $C_9H_{12}BrClN_2O_4$: C, 33.00; H, 3.69; Br, 24.39; Cl, 10.82; N, 8.55. Found: C, 32.95; H, 3.64, Br, 24.63; Cl, 10.69; N, 8.51.

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The crude salt was stirred into a mixture of water, ether, and a slight excess of $NaHCO_3$ to convert it to the aldehyde. After 5 min the layers were separated, and the organic solid was isolated in the usual way. Direct conversion of 1 to 4-bromo-2-pyrrolecarboxaldehyde resulted in yields of 92% (bromination at 28°, 0.5 hr) and 89% (0°, 16 hr), mp 122.5–124.5° (C_6H_6) (lit.[6] mp 123–124°).

Minor amounts of the 5 isomer were identified by glc comparison with the bromination product from 2-pyrrolecarboxaldehyde prepared as described by Anderson and Lee.[6]

4-Acetyl-2-pyrrolecarboxaldehyde.—Acetyl chloride (0.54 ml) was injected into a violet solution of 1.25 g of 1 and 1.47 g of $AlCl_3$ in 25 ml of CH_2ClCH_2Cl at 0°. The resulting brown mixture was kept at 0° for 16 hr. The mixture was poured over crushed ice, and an aqueous solution of 2 g of NaOH was added. After the mixture had been stirred for 10 min, it was acidified (HCl) and extracted continuously with Et_2O (12 hr). The extract was dried (Na_2SO_4) and concentrated to give 0.54 g (77%), mp 139–142° (C_6H_6) (lit.[7b] 136–137°).

4-Acetyl-2-pyrrolecarboxylic Acid.—Silver nitrate (0.94 g) was dissolved in 95 ml of H_2O and added to 190 ml of 1 *N* NaOH. A solution of 0.51 g of 4-acetyl-2-pyrrolecarboxaldehyde in 38 ml of ethanol was added thereto and the resulting mixture was stirred for 0.5 hr. The mixture was filtered, acidified with HCl, and extracted continuously with ether for 6 hr. The extract was dried ($MgSO_4$) and concentrated to give 0.49 g of the acid, mp ~220° dec (lit.[7b] mp 221.5–223° dec).

Registry No.—1, 27521-94-4; 2, 27521-95-5.

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Sterol Metabolism. XIV. Cholesterol 24-Hydroperoxide[1]

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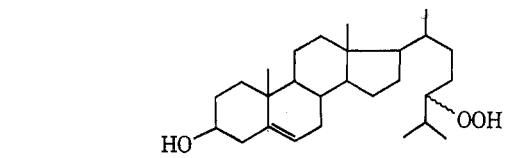
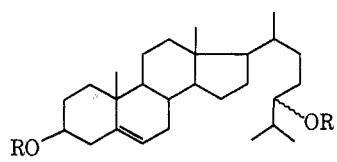
We have isolated from air-aged cholesterol the tertiary hydroperoxides, 3β-hydroxycholest-5-ene 20α-hydroperoxide and 3β-hydroxycholest-5-ene 25-hydroperoxide.[2] A third cholesterol hydroperoxide X_1, previously shown not to be the 17α-hydroperoxide, is identified herein as an epimeric mixture of the 3β-hydroxycholest-5-ene 24-hydroperoxides (I).

Sodium borohydride reduction of the hydroperoxide X_1 gave a mixture of epimeric diols from which one epimer was recovered by crystallization and identified as cholest-5-ene-3β,24ξ²-diol (IIa)[3,4] and from which both cholest-5-ene-3β,24-diol epimers were recovered and identified as their dibenzoates IIb. The 3β,24-diol structure for the cholest-5-ene-3β-24ξ²-diol epimer was suggested by its mass spectrum, which resembled in detail the mass spectra (above *m/e* 200) of the epimeric cholest-5-ene-3β-23-diols.[7] The 3β,24ξ-diols IIa (and their dibenzoates IIb) were distinguished from the known 17α-, 20α-, 22*R*-, 22*S*-, 23*R*-, 23*S*-, 25-, and 25*R*-26-monohydroxylated derivatives of cholesterol but were chromatographically similar to the 3β,24-diol cerebrosterol isolated from human and equine brain.[5] Comparison of the 3β,24ξ²-diol IIa and of the epimeric 3β,24-diol dibenzoates IIb obtained from the hydroperoxide X_1 with authentic sterols established their identity and thereby the identity of the hydroperoxide X_1 as an epimeric mixture of 3β-hydroxycholest-5-ene 24-hydroperoxides (I).

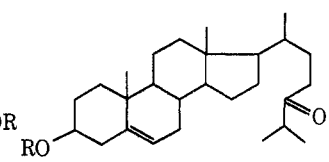
In distinction to the readily acetylated 20α- and 25-hydroperoxides of cholesterol,[2] the 24-hydroperoxides I decomposed on attempted acetylation with acetic anhydride–pyridine. Only 3β-acetoxycholest-5-en-24 one (IIIb) could be identified among the products formed.



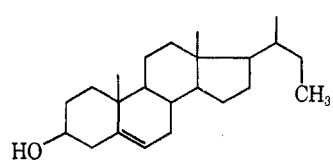
I



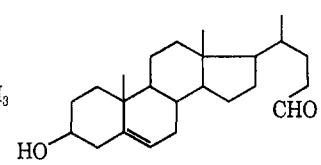
IIa, R = H
IIb, R = Bz



IIIa, R = H
IIIb, R = Ac



IV



V

The instability of the 24-hydroperoxides I to thermal and electron impact degradation was similar to that of the 20α- and 25-hydroperoxides. The three major products previously recognized[8] were identified by their chromatographic and spectral properties as 24-norchol-5-en-3β-ol (IV), 3β-hydroxychol-5-en-24-al (V), and 3β-hydroxycholest-5-en-24-one (IIa). The structure of the alcohol IV as 24-norchol-5-en-3β-ol rests on a consideration of the short gas chromatographic retention times on both 3% QF-1 and 3% SE-30 phases and the relatively high thin layer chromatographic mobility, which data imply a sterol of diminished carbon content. A magenta color with 50%

(1) Paper XIII of the series: J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **49**, 555 (1970). Supported by funds from the U. S. Public Health Service (Grants NS-08106, HE-10160, and AM-13520) and from the Medical Research Council of Canada (Grant MA-4051) and the Conseil de la Recherche Médicale du Québec.

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(3) The original nomenclature for the 3β,24-diols IIa of Ercoli and de Ruggieri[4] is retained: cholest-5-ene-3β,24ξ¹-diol for the epimer named cerebrosterol occurring in human and equine brain,[5] cholest-5-ene-3β,24ξ²-diol for the epimer not found in nature. An absolute stereochemistry as the 3β,24β$_F$ (24*S*)-diol previously assigned[6] the 3β,24ξ¹-diol IIa has been questioned.[7]

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