Regiospecific Attack of Nitrogen and Sulfur Nucleophiles on Quinones Derived from Poison Oak/Ivy Catechols (Urushiols) and Analogues as Models for Urushiol-Protein Conjugate Formation

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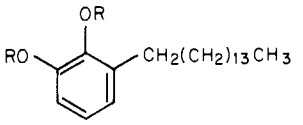
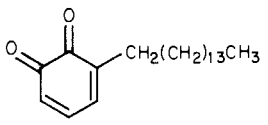
Attempts to characterize potential biologically important covalent interactions between electrophilic quinones derived from catechols present in poison oak/ivy (urushiol) and biomacromolecules have led to the analysis of model reactions involving sulfur and amino nucleophiles with 3-heptadecylbenzoquinone. Characterization of the reaction products indicates that this quinone undergoes regiospecific attack by (*S*)-*N*-acetylcysteine at C-6 and by 1-aminopentane at C-5. The red solid obtained with 1-aminopentane proved to be 3-heptadecyl-5-(pentylamino)-1,2-benzoquinone. Analogous aminobenzoquinones were obtained with the quinones derived from the 4- and 6-methyl analogues of 3-pentadecylcatechol. All three adducts absorbed visible light at different wavelengths. When the starting catechols were incubated with human serum albumin almost identical chromophores were formed. These results establish that catechols responsible for the production of the poison oak/ivy contact dermatitis in humans undergo a sequence of reactions in the presence of human serum albumin that lead to covalent attachment of the catechols to the protein via carbon–nitrogen bonds. Estimations of the extent of this binding indicate that, at least with human serum albumin, the reaction is quantitative.

The commonly encountered plants poison oak (*Toxicodendron diversilobum*) and poison ivy (*T. radicans*) produce catechols bearing at the C-3 straight chain, saturated and unsaturated C_{17} (oak) and C_{15} (ivy) alkyl side chains.[1] These substances, collectively referred to as urushiol, cause a delayed contact hypersensitivity reaction in susceptible individuals.[2] This immune response is likely to be T cell mediated[3] and to require covalent bonding of the catechols to macromolecules.[4] Preliminary evidence from our laboratory indicates that incubation of side-chain tritium-labeled 3-pentadecylcatechol (1) with proteins,



1, R = H
5, R = CH_3



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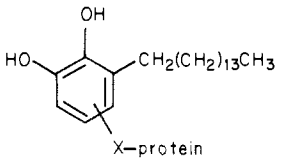

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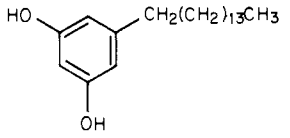

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such as human serum albumin, results in covalent attachment of the catechol to the protein.[5] A likely reaction sequence[6] to account for this binding would involve air-oxidation of the catechol to the corresponding quinone 2, followed by attack of a sulfhydryl or amino nucleophile present on the protein to form adduct 3. The ease of oxidation of catechols[7] and electrophilic properties of *o*-benzoquinones[8] are consistent with this proposal. The lack of antigenic activity of compounds such as 5-pentadecylresorcinol (4) and 3-pentadecylveratrole (5) indicate that the quinone-forming potential of 1 is critical for its ability to cause contact dermatitis.[9,10] The possible relationship between macromolecular adduct formation (conjugation) and the immune response caused by urushiol has prompted us to investigate the chemical reactivity of a series of urushiol type catechols with human serum albumin and the corresponding *o*-benzoquinones with model nucleophiles.

Table I. Absorbance of 10^{-4} M Solutions of Human Serum Albumin (HSA) and 3-Pentadecylcatechol (1)

incubation conditions[a]	time, h				
	4	7	24	48	96
1	0	0	0	0	0
HSA alone	0	0.01	0.04	0.07[b]	
1 and HSA	0	0.02	0.18	0.47	0.49
1, HSA and benzenesulfinic acid	0	0.01	0.03		
1, HSA and O_2	0	0.04	0.36	0.57[c]	
1 and acetylated HSA	0	0	0.003	0.05	

[a] For details of incubation procedures, see text. [b] Absorbance of HSA alone was due to development of turbidity. [c] Not corrected for solvent losses due to evaporation.

Results and Discussion

An earlier study on urushiol–protein conjugate formation[11] reported that solutions of pentadecylcatechol and proteins turned red in color (λ_{max} 480 nm) upon standing in the presence of air. Similar chromophores have been observed for the reaction products of *o*-quinones, generated in situ from the corresponding catechols with tyrosinase, and a variety of amines, amino acids, and proteins.[12] The most likely product which would give rise to such a chromophore would be either an aminoquinone represented by structure 6 or the corresponding iminoquinone. The for-

(1) W. F. Symes and C. R. Dawson, *J. Am. Chem. Soc.*, **76**, 2959 (1954); M. D. Corbett and S. Billets, *J. Pharm. Sci.*, **64**, 1715 (1975); M. Gross, H. Baer, and H. M. Fales, *Phytochemistry*, 14, 263 (1975).
(2) D. Landsteiner and J. Jacobs, *J. Exp. Med.*, **64**, 625 (1936).
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(4) J. J. Arrotti and J. E. Garvin, *Biochem. Biophys. Res. Commun.*, **49**, 205 (1972).
(5) R. G. Dennick, D. J. Liberato, V. S. Byers, N. Castagnoli, Jr., and R. W. Baldwin, unpublished results.
(6) J. S. Byck and C. R. Dawson, *Anal. Biochem.*, **25**, 123 (1968).
(7) J. Cason, *Org. React.*, 4, 305 (1948).
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(9) V. S. Byers, W. L. Epstein, N. Castagnoli, Jr., and H. Baer, *J. Clin. Invest.*, **64**, 1437 (1979).
(10) V. S. Byers, N. Castagnoli, Jr., and W. L. Epstein, *J. Clin. Invest.*, **64**, 1449 (1979).
(11) J. Stauffer, Ph.D. Dissertation, Columbia University (1971).
(12) H. S. Mason and E. W. Peterson, *Biochim. Biophys. Acta*, 111, 134 (1965); W. S. Pierpoint, *Biochem. J.*, 112, 609 (1969).

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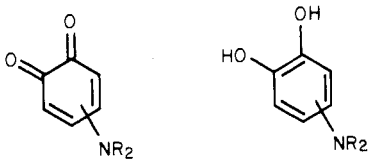

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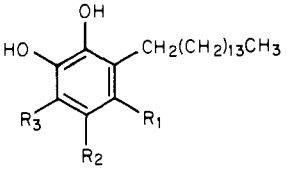
mation of such species presumably would proceed through initial attack by the nucleophile on the *o*-benzoquinone, followed by a two-electron oxidation of the resulting aminocatechol **7**.

The results of studies designed to further characterize the parameters influencing the formation of the chromophore observed in incubations containing equimolar (10^{-4} M) amounts of human serum albumin and 3-pentadecylcatechol are summarized in Tables I and II. The rate of chromophore formation was quite slow. This in part may be due to the low water solubility of 3-pentadecylcatechol and/or the slow rate of chemical reaction(s) leading to the chromophore. Assuming a quantitative reaction, the calculated extinction coefficient for this chromophore is 3136.

The data summarized in Table I are consistent with the proposed quinone pathway. The reaction is facilitated by O_2 and markedly inhibited by the quinone trapping agent benzenesulfinic acid (1.5×10^{-3} M).[13] Additionally, chromophore formation did not take place with human serum albumin which had been acetylated with acetic anhydride. Finally, treatment of the colored solutions with reducing agents such as ascorbic acid and sodium dithionite led to immediate bleaching, presumably because of reduction of the quinoid species to the aminocatechol. With additional stirring in the presence of air, however, the red color reappeared. Consistent with the intermediary role of the quinone **2** in chromophore formation, incubations of 5-pentadecylresorcinol (**4**) and 3-pentadecylveratrole (**5**) with human serum albumin did not result in color formation.

We have extended these studies with a series of analogues which bear methyl groups at the 4, 5, and 6 positions of 3-pentadecylcatechol. Table II summarizes the results obtained by monitoring the visible absorption spectra of incubates of human serum albumin with the analogues. These data establish that the 4- and 6-methylated analogues (**8** and **10**, respectively) also form colored adducts



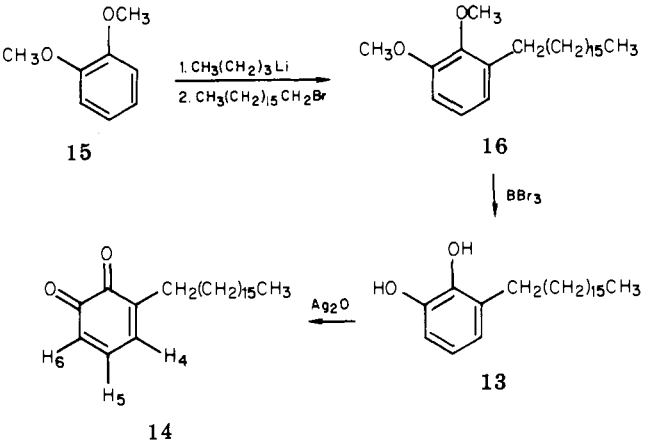
	R_1	R_2	R_3
8	CH_3	H	H
9	H	CH_3	H
10	H	H	CH_3
11	CH_3	CH_3	H
12	CH_3	CH_3	CH_3

with human serum albumin, although in these instances the broad λ_{max} values are shifted to 500–520 and 540–560 nm, respectively. Such bathochromic shifts are consistent with the rules developed by Woodward to predict the influence of alkyl substituents on the electronic absorption spectra of organic molecules.[14] Of considerable interest as well is the observation that the 5-methyl, 4,5-dimethyl, and 4,5,6-trimethyl analogues (**9**, **11**, and **12**, respectively) do not form colored adducts with human serum albumin.

Table II. Absorbance Characteristics Following Incubations of Human Serum Albumin Solutions with the Indicated Analogue of 3-Pentadecylcatechol

analogue	λ_{max} obsd, nm	absorbance at 48 h
8	500–520	0.22
9	no absorbance	
10	540–560	0.24
11	no absorbance	
12	no absorbance	

Scheme I



The results obtained with the protein conjugates suggested to us that the interactions between these catechols and human serum albumin may involve well-defined covalent bonds which could be further characterized by studying the reactions of model nucleophiles with purified *o*-benzoquinones derived from urushiol-type catechols. Because of our interest in poison oak, our initial model studies utilized 3-heptadecylcatechol (**13**) and the corresponding quinone 3-heptadecylbenzoquinone (**14**).

The synthesis of **13** was readily achieved according to the method previously reported by Dawson[15] for the synthesis of 3-pentadecylcatechol. When treated with *n*-butyllithium followed by heptadecyl bromide, veratrole (**15**) underwent selective alkylation at C-3 to form 3-heptadecylveratrole (**16**) (Scheme I). Cleavage of the ether functionalities was achieved with boron tribromide to give a 97% yield of the desired catechol **13**. The ^{1}H NMR spectrum of **13** displayed a sharp singlet at 6.70 ppm integrating for 3 protons. We have observed the same signal for a variety of 3-alkyl substituted catechols and have assigned it to the three aromatic protons.

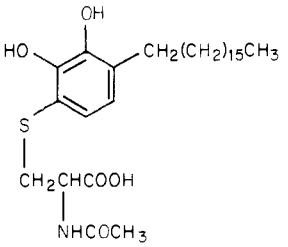
When 3-heptadecylcatechol was treated under mild conditions with freshly prepared silver oxide in diethyl ether,[16] a yellow-greenish colored solution was generated (λ_{max} 408). The ^{1}H NMR spectrum of the material isolated from this reaction is completely consistent with the expected quinone **14**. Based on coupling constants, three well-resolved low-field multiplets centered at 6.6, 7.0, and 6.3 ppm are assigned to the 3-ring protons at C-4, C-5, and C-6, respectively. The ^{1}H NMR spectrum assured us that none of the starting catechol was present in this sample, since no signal was observed at 6.70 ppm.

(13) L. Horner and S. Gowecke, *Chem. Ber.*, **94**, 1267 (1961).
(14) R. B. Woodward, *J. Am. Chem. Soc.*, **63**, 1123 (1941).
(15) C. R. Dawson and G. P. Ng, *Org. Prep. Proced. Int.*, **10**(4), 167 (1978).
(16) R. Willstatter and A. Pfannanstiel, *Ber. Dtsch. Chem. Ges.*, **37**, 4744 (1904).

This *o*-quinone is reasonably stable when stored in the solid state at very low temperature. However, a concentrated solution (>5 mg/mL) upon standing at room temperature undergoes decomposition within a few hours. ^{1}H NMR analysis of such a solution in deuteriochloroform showed the presence of a relatively strong signal at 6.70 ppm which previously had been observed for the catechol starting material. Additionally, silica gel TLC analysis of even freshly prepared quinone displayed a spot which cochromatographs with the starting catechol. Finally, mass spectral analysis of the pure quinone consistently displayed ions corresponding to the starting catechol under a variety (EI, CI, FD) of ionizing conditions. This mass spectral characteristic has been reported previously with various *o*-benzoquinones.[17] The details of the chemical pathway leading to the reduction of the *o*-quinone to the catechol under the various conditions which we have described are not clear. The ^{1}H NMR spectrum of the "decomposed" product showed additional unassignable peaks which suggest that the quinone itself may undergo some type of disproportionation reaction. Further work in this area will be required before it will be possible to rationalize these observations.

With the availability of pure **14**, we examined its reactivity with (*S*)-*N*-acetylcysteine, a model sulfhydryl nucleophile. The reaction, which was monitored by observing the disappearance of the visible chromophore of **14**, proceeded very rapidly even in a dilute (10^{-5} M) 1:1 molar solution of quinone and nucleophile. The chromophore which replaced that of **14** absorbed maximally at 282 nm. It was difficult, however, to measure the formation of the new chromophore quantitatively because of the tendency of **14** to undergo the previously mentioned reduction to the catechol which absorbs in the same region (λ_{max} 275 nm).

When run on a preparative scale, it was possible to isolate a colorless solid which resisted crystallization. Both ^{13}C and ^{1}H NMR allowed us to unambiguously assign the structure of this product as compound **17**. The appear-



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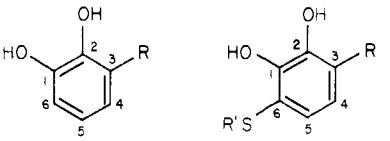
ance of two strongly coupled low-field doublets (6.64 and 6.92 ppm, J = 8.1 Hz) clearly established that the aromatic protons are ortho to one another, thus ruling out the isomer involving thiolation at C-5 of the quinone. In view of the steric hindrance at C-4, it seemed most likely that thiolation of **14** had occurred at C-6.

Careful analysis of the ^{13}C NMR spectra of 3-heptadecylcatechol and the *N*-acetylcysteine adduct confirmed this structure assignment. The signals for all ring carbon atoms of **13** (Table III) could be assigned as shown on the basis of literature reports for analogous compounds.[18] The

Table III. ^{13}C NMR Assignments for Compounds 13 and 17



13 17

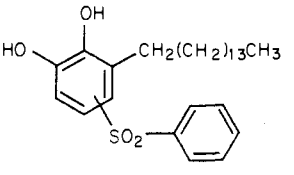
no.	chemical shift, ppm			
	C_1 and C_2	C_3	C_4 and C_5	C_6
	143.89		119.71[a]	
13		129.89		113.36[a]
	145.83		121.76[a]	
	144.31		121.66[a]	
17		131.04		118.03
	146.35		125.60[a]	

[a] When T_1 relaxation experiments were conducted, these resonances displayed signal enhancement attributable to directly bonded hydrogen atoms.

introduction of an alkylthio group would be expected to cause a downfield shift of the ring carbon atom signals. The most dramatically altered signal observed is that assigned to C_6 of **13**, which shifted from 113.36 to 118.03 ppm. Any other assignment would involve an unlikely upfield shift of one of the remaining signals to 118.03 ppm. T_1 relaxation studies confirmed that this carbon atom does not bear a bond to hydrogen. Based on these data, we have assigned the site of attachment of the alkylthio group to C_6.

Additional evidence for this assignment was obtained by analyzing the reactivity of the various monomethylated compounds in the 3-pentadecylcatechol series. In each instance the methyl-substituted catechol was converted to the corresponding quinone, which was then treated with (*S*)-*N*-acetylcysteine. The reaction mixture was monitored by absorption spectroscopy. The results showed that the quinones derived from **1** and its 4- and 5-methylated analogues (**8** and **9**, respectively) undergo reaction with (*S*)-*N*-acetylcysteine (replacement of visible chromophores with a UV chromophore absorbing maximally near 284 nm), whereas the 6-methyl analogue (**10**) resisted this reaction (no loss of the visible chromophore).

We previously had observed that benzenesulfinic acid inhibited the covalent binding of radiolabeled 3-pentadecylcatechol to proteins.[5] Therefore, we have examined the reaction of 3-pentadecylbenzoquinone (**2**) with benzenesulfinic acid in order to determine if this inhibition might be due to the trapping of the in situ generated quinone intermediate **2**. When a solution of **2** was treated with benzenesulfinic acid, the lime-green color of the quinone solution immediately disappeared. Column chromatography of the reaction mixture, followed by crystallization of the slightly off-colored isolate, provided a pure material which was characterized as **18** by ^{1}H NMR



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and elemental analysis. Because of extensive overlap of the signals in the aromatic region, it was not possible to assign the site of attachment of the benzenesulfonyl group.

(17) J. Heiss, K. P. Zeller, and A. Rieker, *Org. Mass Spectrom.*, **2**, 1325 (1969); S. Ukai, K. Hirose, A. Tatematsu, and T. Goto, *Tetrahedron Lett.*, **49**, 4999 (1967).

(18) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra, A Collection of Assigned, Coded, and Indexed Spectra", Wiley, New York, 1972; E. Breitmaier, G. Haas, and W. Voelter, "Atlas of Carbon-13 NMR Data", Plenum, New York, 1976.

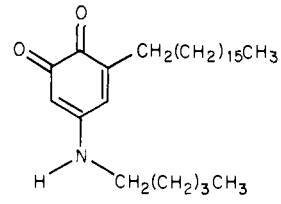
Table IV. Visible Absorption Characteristics of Reaction Mixtures of 1-Aminopentane and Various Substituted o-Benzoquinones

quinone derived from	λ_{max} obsd, nm
13	480-486
8	484-494
9	none
10	540-560

When we examined the reactivity of 3-heptadecylbenzoquinone with the model primary amine 1-aminopentane under reaction conditions similar to those used for (*S*)-*N*-acetylcysteine, no evidence of reaction was observed. However, in the presence of a 1000 M excess of the amine, a red color formed which proved to have the same λ_{max} as that observed with incubates of 3-pentadecylcatechol and human serum albumin. This observation prompted us to examine this reaction with the quinones derived from the methyl analogues of **1** and to compare the corresponding spectra with those obtained with incubates of these catechols and human serum albumin. Summarized in Table IV are the salient results obtained from this study. Completely analogous behavior was observed with 1-aminopentane and the synthetic quinones derived from these catechols as was observed with incubates of the catechols and human serum albumin (see Table II). The apparent lack of reactivity of the quinone derived from the 5-methyl analogue **9** was particularly noteworthy and suggested that the site of attack of the amino group was at C-5.

These results prompted us to attempt to characterize the reaction product between the model nucleophile 1-aminopentane and 3-heptadecylbenzoquinone (**14**). When performed on a preparative scale, the reaction between 1-aminopentane (100 M excess) and **14** yielded a mixture of products from which 3-heptadecylcatechol and a red-colored solid could be isolated. Although it was not possible to obtain a crystalline material, the red-colored product isolated by preparative TLC proved to be a stable material which could be analyzed by NMR, mass spectrometry, and absorption spectroscopy. The EI and CI mass spectra of this material showed parent ions expected for a quinoid structure, but, in addition, displayed ions consistent with a reduced aminocatechol structure. Based on the NMR characteristics (see below), we feel that the mass spectral behavior of this product is likely to be analogous to that previously observed with **14** which, under mass spectral conditions, undergoes reduction to generate ions 2 mass units higher than the product itself.

The ^1H NMR spectrum of the reaction product formed from 1-aminopentane and **14** displayed two nonexchangeable low-field signals at 6.78 and 5.51 ppm, both of which were broad singlets. This information clearly allowed us to assign the position of attack on the original quinone as that of C-5, since attack at C-4 or C-6 would have led to the presence of a product displaying ortho coupling of the low-field signals. A very broad signal which was centered at 3.32 ppm was assigned to the protons of the methylene carbon atom attached to the nitrogen atom. When the $CDCl_3$ solution was treated with a drop of D_2O, this signal resolved into a well-defined triplet, $J = 6.9$ Hz. The treatment with D_2O also caused the disappearance of a broad signal centered at 7.70 ppm, which we now assign to an NH proton resonance. These results clearly allow us to establish the structure of this product as the *o*-quinone tautomer (structure **19**) as opposed to the iminoquinone tautomer (structure **20**). When a CH_2Cl_2 solution of **19** was shaken with an aqueous solution of sodium dithionite, the red color was immediately discharged. Acetylation of the resulting reduction product **21** with acetic anhydride and pyridine provided the triacetyl derivative **22**, the structure of which was confirmed by high-resolution mass spectral analysis.



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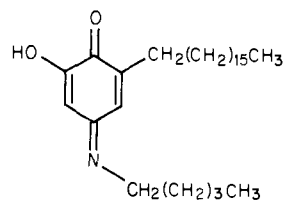

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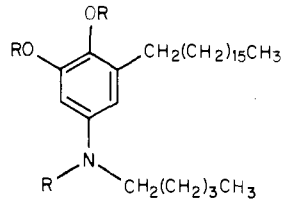

21, R = H
22, R = $COCH_3$

Summary

Results obtained in this study clearly establish that the quinone **14** undergoes regiospecific attack by model sulfhydryl and amino nucleophiles. The factors which determine the different sites of attack of these nucleophiles are not evident. We have observed similar types of specificity with respect to nucleophilic attack by various sulfhydryl functionalities on the quinone derived from 4-methylcatechol.[19] Further work on the mechanisms of these reactions appears to be well-justified.

The data presented in this paper also support the hypothesis that the catechol components of urushiol from covalent adducts with macromolecules by way of initial oxidation to the reactive quinone species, followed by attack of nucleophilic amino functionalities present on proteins. The analogy between the model chemistry observed with the quinones derived from the methylated 3-pentadecylcatechol analogues and 1-aminopentane and the corresponding adducts formed between these analogues and human serum albumin lends very strong support to the thesis that amino functionalities (presumably ϵ-amino groups of lysine residues) attack the in situ generated *o*-quinone to form initially aminocatechols, which then spontaneously oxidize to aminoquinones. The extinction coefficient of the aminoquinone **19** (3236) compares favorably with the apparent extinction coefficient (3136) determined for the reaction product obtained after incubating an equimolar mixture of 3-pentadecylcatechol and human serum albumin. Consequently, at least with human serum albumin under these reaction conditions, amine adduct formation with 3-pentadecylcatechol appears to be quantitative. The importance of sulfhydryl adduct formation with urushiol in biologic systems, however, should not be disregarded, particularly since the *o*-benzoquinones examined in this study are much more reactive toward sulfur nucleophiles than amino nucleophiles. The ratio of amino to sulfhydryl groups present on human serum albumin is near 100.[20] This preponderance of amino

(19) C. G. Chavdarian and N. Castagnoli, Jr., *J. Med. Chem.*, **22**, 1317 (1979).
(20) A. R. Goldfor, *Biochemistry*, **5**, 2574 (1966); R. E. Benesch, H. Lardy, and R. Benesch, *J. Biochem. (Tokyo)*, **216**, 663 (1955).

groups is likely to account for the efficient trapping of the in situ generated quinones by nitrogen nucleophiles. The biological significance of urushiol–protein adduct formation may become more apparent when these studies are extended to include the characterization of the processing of these catechols by immunocompetent cells.

Experimental Section

Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded with a Varian FT-80 instrument. Chemical shifts are reported in parts per million (δ) relative to Me_4Si. IR spectra were obtained on a Perkin-Elmer 457 spectrophotometer and absorption spectra on a Cary 118. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

3-Heptadecylveratrole (16). To a solution of veratrole (18 g, 130 mmol) in 300 mL of dry THF (sodium and benzophenone distilled) in a 1-L three-neck round-bottom flask fitted with a reflux condenser, dropping funnel, and septum was added over a 30-min period under N_2, with stirring and ice cooling, 42 mL (88 mmol) of a 2.1 M *n*-butyllithium solution in hexane. The thick yellow suspension which formed was stirred at 0 °C for 2.5 h and then was heated under reflux for 0.5 h. A solution of 16 g (50 mmol) of 1-bromoheptadecane (Eastman) in 20 mL of THF was then added over a 15-min period to the stirring ice-cooled yellow solution. The reaction mixture turned milky white after 0.5 h at room temperature and was warmed to reflux for 17 h. After cooling in an ice bath, the clear straw brown solution was treated carefully with 50 mL of 10% HCl. The resulting mixture was allowed to separate, and the aqueous layer was extracted with 250 mL of ether. The combined organic phases were first washed with 3 × 50 mL of 10% NaOH and then with saturated aqueous NaCl. After drying over $MgSO_4$ and evaporating the solvent, the resulting yellow oil was distilled. The 150–190 °C (0.25 mm) boiling fraction solidified and was recrystallized from 95% EtOH to yield 7.67 g (41%) of 3-heptadecylveratrole: mp 43–44 °C; ^1H NMR ($CDCl_3$) δ 0.87 (m, 3 H, CH_3), 1.25 (s, 28 H, 14-CH_2), 1.53 (m, 2 H, Ar CH_2CH_2), 2.62 (m, 2 H, Ar CH_2), 3.80 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 6.82 (m, 3 H, Ar H); UV (EtOH) λ_{max} 271 nm (log ϵ 3.07), 277 (3.06). Anal. ($C_{25}H_{44}O_2$) C, H, N.

3-Heptadecylcatechol (13). To 1 g (2.66 mmol) of **16** in 40 mL of methylene chloride was added dropwise, with stirring and ice cooling under N_2, 2 g (8 mmol) of boron tribromide. After 1 h, the reaction mixture was allowed to warm to room temperature and was stirred for an additional 24 h. The mixture was then cooled to 0 °C and 10 mL of anhydrous methanol was added. After the solvent was removed on a rotor evaporator, the residue was treated with an additional 20 mL of methanol. The solvent was again removed, and the residue was subjected to bulb to bulb distillation [184 °C (0.4 mm)], which yielded 900 mg (97%) of 3-heptadecylcatechol: mp 62.5–64 °C (lit.[21] mp 62–63 °C); ^1H NMR ($CDCl_3$) δ 0.87 (m, 3 H, CH_3), 1.25 (s, 28 H, 14-CH_2), 1.60 (m, 2 H, Ar CH_2CH_2), 2.66 (m, 2 H, Ar CH_2), 5.08 (br s, 2 H, 2-OH), 6.70 (s, 3 H, ArH); decoupled ^{13}C NMR ($CDCl_3$) δ 145.03 (13 relative intensity, *C*OH), 143.89 (11, *C*OH), 129.89 (13, C_3), 121.76 (62, C_4 or C_5), 119.71 (65, C_4 or C_5), 113.36 (63, C_6), 32.57 (50, Ar CH_2), 30.35 (512, 14-CH_2), 23.24 (46, CH_2CH_3), 14.30 ppm (42, CH_3); UV (CH_2Cl_2) λ_{max} 275 nm (log ϵ 3.32); IR (CCl_4) 3620, 3575, 2940, 2870, 1485, 1360, 1285 cm^{-1}; CIMS (isobutane) MH^+ 349.

3-Heptadecylbenzoquinone (14). To 3.32 g (14.34 mmol) of freshly prepared silver oxide in 150 mL of dry Et_2O was added 5 g of sodium sulfate, followed by 250 mg (0.72 mmol) of **13** dissolved in 100 mL of Et_2O. The flask was shaken for 4 min and filtered over Celite, and the solvent was removed at 0 °C to yield 240 mg (97%) of a yellow-green solid: mp 68–70 °C dec; ^1H NMR ($CDCl_3$) δ 0.87 (m, 3 H, CH_3), 1.25 (s, 30 H, 15-CH_2), 2.36 (m, 2 H, Ar CH_2), 6.25 (dd, $J_{5,6}$ = 9.8 Hz and $J_{4,6}$ = 1.7 Hz, 1 H, H_6), 6.74 (m, 1 H, H_4), 6.99 (dd, $J_{5,6}$ = 9.8 Hz, $J_{4,5}$ = 6.3 Hz, 1 H, H_5); UV (CH_2Cl_2) λ_{max} 268 nm sh (log ϵ 3.11), 408 (3.23); IR (CCl_4) 2940, 2860, 1690, 1665, 1400 cm^{-1}. Anal. ($C_{23}H_{38}O_2$) C, H, N.

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The preparations of quinones derived from 3-pentadecylcatechol (1) and its analogues followed the above procedure.

***N*-Acetyl-*S*-(2,3-dihydroxy-4-heptadecylphenyl)cysteine (17).** To an ice-cooled solution of 995 mg (2.87 mmol) of **14** in 500 mL of dry THF under N_2 was added dropwise over 30 min 515 mg (3.16 mmol) of (*S*)-*N*-acetylcysteine in 100 mL of dry THF. After an additional 45 min of stirring at 0 °C, the reaction mixture was allowed to warm to room temperature and was stirred an additional 45 min. The solvent was evaporated and the resulting yellow oil was dissolved in 250 mL of Et_2O, which was extracted 3 × 50 mL of H_2O and dried with $MgSO_4$. The oil obtained after removing the solvent was then subjected to silica gel column chromatography (LoBar LiChroprep Si 60). Elution with ethyl acetate/methylene chloride/acetic acid (25:25:4) gave 561 mg (38%) of a white solid: ^1H NMR ($CDCl_3$) δ 0.87 (m, 3 H, CH_2CH_3), 1.25 (s, 30 H, 15-CH_2), 1.91 (s, 3 H, O=CCH_3), 2.59 (br t, 2 H, Ar CH_2), 3.20 (br m, 2 H, SCH_2), 4.80 (br s, 1 H, CH), 6.88 (br m, 5 H, 2 Ar H, 2 OH, 1 NH); ^1H NMR ($CDCl_3$ + D_2O) δ 0.87 (m, 3 H, CH_2CH_3), 1.25 (s, 30 H, 15-CH_2), 2.59 (br t, 2 H, Ar CH_2), 3.22 (br m, 2 H, SCH_2), 4.72 (br t, 1 H, CH), 6.64 (d, J = 8.1 Hz, 1 H, 1 Ar H), 6.92 (d, J = 8.1 Hz, 1 H, 1 Ar H); decoupled ^{13}C NMR ($CDCl_3$) 172.84 (7 relative intensity, *C*=O), 170.71 (7, *C*=O), 146.35 (7, *C*OH), 144.31 (8, *C*OH), 131.04 (9, C_3), 125.60 (12, C_4 or C_5), 121.66 (12, C_4 or C_5), 118.03 (6, C_6), 53.34 (9, CCH_2S), 32.41 (13, Ar CH_2), 30.19 (130, 14-CH_2), 28.82 (15, CH_2S), 23.1 (11, $HNCH_3$ or CH_2CH_3), 22.71 (10, $HNCH_3$ or CH_2CH_3), 14.25 ppm (11, CH_2CH_3); UV (EtOH) λ_{max} 254 nm (log ϵ 3.63), 288 (3.34). High-resolution MS for $C_{28}H_{47}NO_5S$ (M^+): calcd, *m/e* 509.31750; found, *m/e* 509.314697. Anal. Calcd for $C_{28}H_{47}NO_5S$: C, 65.98; H, 9.49; N, 2.74. Found: C, 65.07; H, 8.95; N, 2.89.

3-Pentadecyl-5-(phenylsulfonyl)catechol (18). To 103 mg (0.324 mol) of freshly prepared 3-pentadecylquinone (**2**) in 50 mL of dry ether was added, with stirring, 60 mg (0.365 mmol) of benzenesulfinic acid in 0.5 mL of acetic acid. The lime-green color of the reaction mixture disappeared immediately. The solution was evaporated to yield a tan solid, which was purified by column chromatography (silica gel 60, 70–230 mesh; heptane/ethyl acetate/acetic acid, 50:50:1) and recrystallized from hexane–ether to yield 128 mg (0.28 mmol, 86%) of a beige solid: mp 96.5–98 °C; ^1H NMR ($CDCl_3$) δ 0.87 (m, 3 H, CH_3), 1.25 (m, 26 H, 13-CH_2), 2.62 (t, 2 H, Ar CH_2), 7.53 (m, 7 H, Ar H); IR (KBr) 3440, 3325, 2920, 2850, 1325, 1280, 849, 755, 720 cm^{-1}. Anal. ($C_{27}H_{40}O_4S$) C, H, N.

3-Heptadecyl-5-(pentylamino)-1,2-benzoquinone (19). To a solution of 99.4 mg (0.29 mmol) of quinone **14** in 100 mL dry CH_2Cl_2 was added 2.5 g (28.7 mol) 1-aminopentane. The flask was shaken for 4 min and then diluted with an additional 150 mL of CH_2Cl_2. The reaction mixture was then washed with 3 × 50 mL of 1 M KH_2PO_4 (pH 3) and 1 × 50 mL of brine, dried over $MgSO_4$, filtered, and evaporated at 0 °C. Preparative TLC (silica gel, ethyl acetate/acetic acid, 25:1) gave a red band near the origin, which was eluted first with CH_2Cl_2 and then EtOAc to yield 40 mg (32%) of a red solid: mp 113–114 °C dec; ^1H NMR ($CDCl_3$) δ 0.87 (m, 6 H, 2-CH_3), 1.25 (s, 36 H, 18-CH_2), 2.36 (br m, 2 H, Ar CH_2), 3.32 (br s, 2 H, NCH_2), 5.51 (br s, 1 H, CH), 6.78 (br s, 1 H, CH), 7.68 (br s, 1 H, NH); ^1H NMR ($CDCl_3$ + D_2O) δ 0.87 (m, 6 H, 2-CH_3), 1.25 (s, 36 H, 18-CH_2), 2.36 (br m, 2 H, Ar CH_2), 3.32 (t, J = 6.9 Hz, 2 H, NCH_2), 5.51 (br s, 1 H, CH), 6.78 (br s, 1 H, CH); UV (CH_2Cl_2) λ_{max} 293 nm (log ϵ 4.03), 480 (3.51); IR ($CDCl_3$) 3390, 3200, 2890, 2835, 1675, 1640, 1600, 1550, 1450, 1350 cm^{-1}. High-resolution MS for $C_{28}H_{49}NO_2$ (M^+): calcd, *m/e* 431.37634; found, *m/e* 431.37495. For reduced species $C_{28}H_{51}NO_2$ (M^+): calcd, *m/e* 433.3920; found, *m/e* 433.39326. For the triacetyl derivative $C_{34}H_{57}NO_5$ (M^+): calcd, *m/e* 559.423687; found, *m/e* 559.420786.

Incubation of Human Serum Albumin with 3-Pentadecylcatechol and Analogues. These catechols are essentially insoluble in water. Therefore, in order to study their interactions with human serum albumin, 1 mL of an acetone solution containing 1.56 μmol of **1** or analogue was transferred to a 50-mL Erlenmeyer flask. After the solvent was removed under a stream of N_2, 10 mL of a pH 7.0 buffered phosphate solution (0.01 M) containing 100 mg (1.50 μmol) human serum albumin was added. The resulting heterogeneous mixtures were stirred open to the air at room temperature, during which time the catechols slowly dissolved. The solutions were periodically examined spectro-

scopically and the positions and intensities of the visible chromophores recorded (see Tables I and II).

Acetylation of human serum albumin was achieved by adding 20 mg (194 mmol) of acetic anhydride to a stirring solution of 200 mg (3×10^{-3} mmol) of human serum albumin in 40 mL of 0.01 M pH 7.0 phosphate buffer. After stirring for 1.5 h at room temperature, the solution was dialyzed against 2 L of distilled water overnight, after which the protein solution was lyophilized.

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Permethyl Analogue of the Pyrrolic Antibiotic Distamycin A

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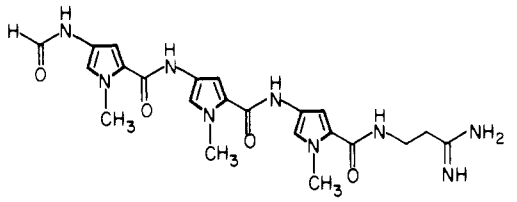
The synthesis of an analogue of distamycin A, a pyrrolic oligopeptide possessing antiviral and antibiotic activity, is described in which each of the three pyrrole rings is fully methylated. This structural modification results in pyrrole rings which are extraordinarily electron rich and required the development of a new synthetic approach to these polypyrrolic amides. The key reactions involved development of a general method for the synthesis of 3-aminopyrroles and for formation of an amide bond between a pyrrole-2-carboxylic acid and these 3-aminopyrroles. Since the acid is hindered, a poor electrophile, and acid sensitive, while the amine is unstable and a hindered, weak nucleophile, amide bond formation under the usual conditions was poor. A very efficient method, however, was developed involving the isolation of 1-hydroxybenzotriazole active ester prepared in situ from another active ester. Neither the mono-, di-, nor tripyrrolic permethyl analogues were effective antimalarials, and none showed anticancer activity.

Distamycin A (1), a naturally occurring substance isolated from *Streptomyces distallicus*,[1] has been the subject of numerous chemical[1-6] and biological investigations.[7,8] It has antiviral, antitumor, and antibacterial activity and forms complexes with various types of DNA, thus inhibiting DNA-dependent syntheses.[7] It is a member of a modest class of oligopeptides, all of which are biologically active; this class includes anthelvencin A (2),[9] congocidin (3),[10] and kikumycin B (4),[11] which contain a common pyrrole subunit, and amidinomycin (5)[12] and noformycin (6),[13] which are nonaromatic. Their biological activity has stimulated much work in several fields, including molecular biological endeavors, since the interactions of distamycin



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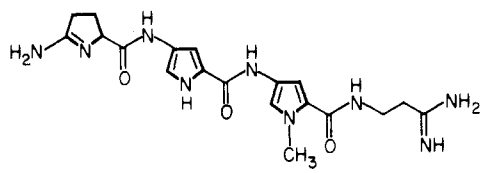

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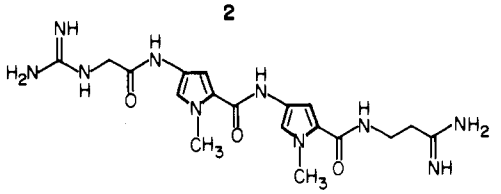

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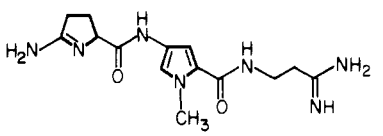

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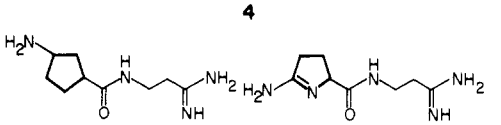

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A and congocidin with DNA reveal substantial information about the DNA. This, in turn, has led to syntheses of two (1 and 3) of the natural products,[2,6a,10] and the syntheses of homologues and analogues[3-5,6b,8,14-16] soon followed.

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