Jatrophone, Jatrophone Derivatives, and Analogues. Conformation and Reactions with Propanethiol

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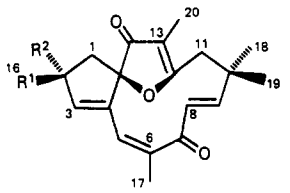
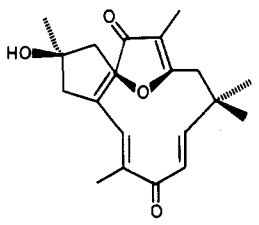
An investigation of the reactions of jatrophone, its derivatives, and synthetic analogues with propanethiol, a model biologic nucleophile, is described. This work, which expands the earlier study of Kupchan on the reaction of jatrophone with propanethiol, was initiated to determine what effect, if any, the solution conformation of these derivatives would have on the nature of the derived products. Toward this end, conformations were determined in the solid state by X-ray crystallography and in solution via nuclear Overhauser enhancement studies. No significant differences between the crystal and solution conformations were observed. Reactions were then carried out with propanethiol, products isolated, and the structures assigned. The results indicate that the conformation of the macrocyclic ring does not have a profound effect on the nature of the products. However, the observation that the principal product of thiol addition is a bisadduct is significant in terms of the chemical mechanism of the cytotoxic effect in that it suggests that cross-linking may play an important role.

Introduction

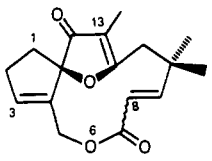
The susceptibility of natural products containing α,β-unsaturated carbonyl functions to conjugate addition of alkanethiols is often employed as a significant indicator of potential cytotoxicity.[2,3] The α-methylene γ-lactones such as elephantin[4] and vernolepin[5] are well-known examples. Less reactive compounds containing endocyclic α,β-unsaturated carbonyls such as the eremantholides[6] and jatrophone (**1**)[7] are nonetheless highly cytotoxic. In each case conjugate addition of alkanethiols to these compounds has been demonstrated in vitro.[2-7] It is assumed that these systems serve as electrophiles by reacting with endogenous thiol groups present on important enzymes, resulting in enzyme dysfunction and thereby cell death.[2,3]



(**1**) R^1 = H, R^2 = CH_3
(**2**) R^1 = CH_3, R^2 = OH
(**3**) R^1 = OH, R^2 = CH_3
(**4**) R^1 = R^2 = H



(**5**)



(**6**) *trans*
(**7**) *cis*

Recently we reported the structures of three new, natural derivatives of jatrophone, which we termed hydroxyjatrophones A, B, and C (**2**, **3** and **5**).[8] In addition, several unnatural isomers and derivatives of jatrophone such as 2-normethyljatrophone (**4**)[9] and the jatropholactones **6** and **7**[10,11] have been prepared in the course of our recently completed total synthesis of jatrophone (**1**).[9]

In this, a full report, we detail an investigation of the reaction of jatrophone, its derivatives, and analogues with propanethiol. This work expands the earlier study of Kupchan on the reaction of jatrophone itself with propanethiol,[7b] in which it was reported that treatment with excess propanethiol under basic conditions (pH 9.2) led to a single adduct. This adduct, assigned structure **8** on spectroscopic evidence, apparently resulted from conjugate addition of propanethiol to the α,β-unsaturated carbonyl system followed by, or in concert with, a novel transannular bond formation between C(8) and C(12). An analogous process occurs when jatrophone is treated with mineral acids. In this case however a bis adduct (**9**) is formed, containing two new transannular bonds (X = Cl or Br). The structure of the dibromo adduct **9**, X = Br, initially deduced through analysis of the spectral data, was confirmed by X-ray crystallographic analysis.[7a] Our motivation in expanding the Kupchan study was to determine what effect, if any, the solution conformation of the above derivatives had on the course of the addition-cyclization.

Background

The remarkable similarity of the solution conformations of the jatrophones **1-4**, as revealed via nuclear Overhauser enhancement (NOE) studies, to the crystal conformations

(1) (a) National Institutes of Health Postdoctoral Fellow, 1981-1983. (b) Camille and Henry Dreyfus Teacher-Scholar, 1978-1983; National Institutes of Health (National Cancer Institute) Career Development Awardee, 1980-1985.

(2) (a) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* **1971**, *14*, 1147. (b) Kupchan, S. M.; Fessler, D. C.; Eakin, M. A.; Giacobbe, T. J. *Science (Washington, D.C.)* **1970**, *168*, 376. (c) Kupchan, S. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1974** *33*, 2288.

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(10) Malamas, M. S.; Smith, A. B., III, *J. Org. Chem.* **1982**, *47*, 3442.

(11) The jatropholactones **6** and **7** were tested against Hela cells by T. Takita of Microbial Chemistry, Tokyo. They were found to have IC_{50} values of 25 and 100 μg/mL, respectively. Jatrophone (**1**) was very toxic at the lowest dose tested, 1.56 μg/mL.

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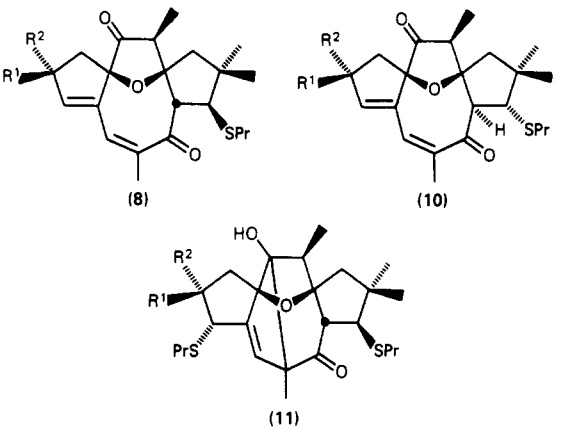
as determined for jatrophone (1)[7] and 2-normethyljatrophone[9] (4) has recently been discussed in detail.[8] In brief, the macrocyclic ring is folded underneath the furanone ring with the carbonyl and C(8,9) double bond in a transoid conformation in a plane parallel to the furanone ring. The C(5,6) double bond is essentially orthogonal to the macrocyclic ring carbonyl. The solid-state and solution conformations of isojatrophone **5** were again indistinguishable but notably different from 1.[8] In this case, the macrocyclic ring is slightly puckered but forms a nearly planar array that is perpendicular to the furanone ring.

Our previous NOE studies have now been extended to the synthetic jatrophone analogues, jatropholactones **6** and **7**. Dreiding-type molecular models of *trans*-jatropholactone (**6**), for example, indicate that a conformation similar to jatrophone is present, in that the C(7) carbonyl and the C(8,9) olefin form a plane parallel to the furanone ring. In the model, at least, **6** is more flexible than **1**; either a cisoid or transoid conformation of the macrocyclic ring carbonyl and double bond may exist. That the former predominates in solution is supported by NOE studies. In particular, irradiation of the upfield doublet of the C(5) methylene resonance at δ 4.27 produced a 20.7% enhancement of the C(8) olefinic resonance, whereas irradiation of the downfield doublet produced only an enhancement of the C(3) olefinic resonance. These results require a cisoid conformation of the α,β-unsaturated system. In this conformation, the C(8) olefinic proton is proximal to the C(5)α proton and the C(5)β proton is proximal to C(3). A single-crystal X-ray analysis of **6** has now been completed, and one again finds the solid-state and solution conformations to be essentially identical. In this case, the C(7) carbonyl and C(8,9) olefin are ca. 45° from coplanarity but the overall conformation is indeed cisoid.[12]

The model of *cis*-jatropholactone (**7**) on the other hand reveals that the macrocyclic ring is fairly rigid. Furthermore, the C(8,9) double bond can only be forced into a coplanar orientation with the lactone carbonyl with considerable difficulty. The relatively small chemical shift difference for the C(8,9) olefinic protons (0.14 ppm) reflects this fact.

Results

Preliminary to our comparative study of the reactions of the six natural and synthetic derivatives of jatrophone (i.e., **2**–**7**) with propanethiol, we reexamined the reaction of jatrophone itself. In this experiment, treatment of jatrophone with propanethiol under the identical conditions described by Kupchan[7b] produced not one but three adducts. One of these products was assigned structure **8a** on the basis of its spectroscopic properties (vide infra). The NMR spectrum of this derivative, however, did not correspond to the data reported for the adduct obtained by Kupchan, which had been assigned the same structure. The two minor adducts (**10a** and **11a**) also did not appear to correspond to the Kupchan adduct.[13]



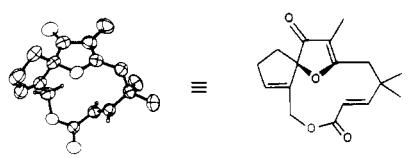
(a) R^1 = H, R^2 = CH_3 (c) R^1 = OH, R^2 = CH_3
(b) R^1 = CH_3, R^2 = OH (d) R^1 = R^2 = H

In our hands the three products **8a**, **10a** and **11a** were formed in a ratio of 4:1:1 after a 30-min reaction period. Monoadducts **8a** and **10a** were inseparable by thin-layer chromatography. The bisadduct, on the other hand, was easily separated from the monoadducts by preparative thin-layer chromatography. Assignment of structure and stereochemistry of these adducts proceeded as follows. The slow-moving product (**11a**) had a molecular formula determined by high-resolution mass spectrometry to be $C_{26}H_{40}O_3S_2$. The infrared spectrum exhibited absorptions consistent with the presence of an alcohol (3650–3200 cm^{-1}) and a carbonyl function (1705 cm^{-1}), while a single olefinic resonance was observed in the 250-MHz NMR spectrum at δ 5.32. In addition to two triplets attributable to the propyl thioethers, five other methyl resonances were observed, two were doublets and three were singlets. The methyl singlet at lowest field (δ 1.30) was not sufficiently deshielded to be consistent with a vinyl methyl group. Taken together these observations allow assignment of the carbon skeleton indicated by structure **11a**.

The stereochemistry of **11a** was deduced from the observed coupling constants. Towards this end, a Dreiding molecular model study of the various possible stereochemical combinations was performed, the resulting dihedral angles were measured, and the magnitude of the coupling constants was predicted[14] and compared with observed values. A β-configuration of the thioether at C(3) results in a predicted value for J_{2H-3H} of 2 Hz (θ = 95°), while an α-configuration results in a predicted value of 8 Hz (θ = 28°). The latter corresponds closely to the observed coupling of 7.7 Hz. This C(3)α configuration, it may be noted, is in contrast to the C(3)β configuration assigned via single-crystal X-ray analysis to the bis(hydrobromide) adduct **9**. A similar analysis requires that the large coupling constant (12.4 Hz) observed for J_{8H-9H} be the result of a trans configuration. Finally, the β configuration of the C(9) thioether was assigned on the basis of conformational and mechanistic arguments (vide infra).

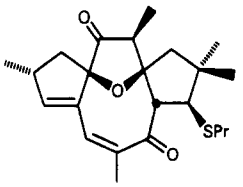
While the mixture of monoadducts **8a** and **10a** was not separable chromatographically, the NMR spectrum of this mixture clearly indicated, by integration of the olefinic

(12) Unpublished results of P. Carroll and V. Santo Pietro of the University of Pennsylvania. The details of this study will be reported elsewhere as part of a larger investigation of the crystal structures of jatrophone derivatives and analogues. An ORTEP plot of the crystal structure of **6** is illustrated below.



(13) We are grateful to Professor A. Sneden of Virginia Commonwealth University for his efforts in searching for spectra of the Kupchan *n*-propanethiol–jatrophone adduct (**8**). Without benefit of the original spectrum of this adduct and given the limited data reported,[7b] we are unable to determine whether the discrepancy results from an error in reporting of chemical shift data or a substantive difference in products observed. We suspect the former.

(14) Becker, E. D. "High Resolution NMR"; Academic Press: New York, 1969; pp 103–4.



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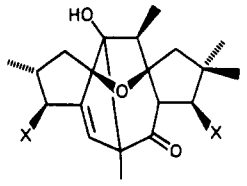


(9)

region, two components in a ratio of 4:1. Two carbonyl absorbances at 1740 and 1660 cm^{-1} were also observed in the infrared spectrum. Upon resubmission of the mixture to the same reaction conditions, the major adduct **8a** was completely converted in less than 2 h to the bisadduct **11a**. The minor adduct remained unchanged and could then be isolated, purified, and characterized. Furthermore, an NMR spectrum of the major monoadduct **8a** was obtained when the spectrum of **10a** was subtracted electronically from the spectrum of the mixture. Both spectra were consistent with monopropanethiol adducts. That **10a** was in fact a monoadduct was confirmed via high-resolution mass spectroscopy, which indicated a molecular formula of $C_{23}H_{32}O_3S$. In addition, two signals consistent with olefinic protons were observed for each adduct as well as two methyl doublets and three singlets. One of the singlets was observed at relatively low field in each derivative (δ 1.95 in **10a**, δ 1.88 in **8a**), indicating a vinyl methyl group. That in both cases thiol addition had occurred at C(9) and not at C(3) derives from the observed loss of the AB system attributable to the C(8,9) olefinic protons as well as retention of the characteristic pattern of resonances for the cyclopentene ring protons. The fact that the C(8) and C(9) protons were trans was deduced from the magnitude of the coupling constants (12.8 and 13.1 Hz in **10a** and **8a**, respectively).

The remaining structural problem of **8** and **10** concerned the assignment of the relative configuration at C(8) and C(9). Since a direct determination was not possible, our assignment is based on mechanistic grounds. Again Drieding-type models of **8** and **10** were prepared as well as intermediates that would be formed in the conversion of **8a** to **11a** and **10a** to its corresponding bis(propanethiol) adduct. The intermediate dienolate formed by addition of propanethiol to C(3) of **8a** was found in the model to be relatively strain free. Furthermore, C(6) was only 2.9 Å from the C(14) carbonyl carbon and thus close to bonding distance. The model of the corresponding enolate derived from **10** could not be made, thereby indicating a large strain energy inherent in such a structure. This observation is consistent with the inertness of **10** to further thiol addition. On the basis of this analysis we assigned the C(9)β configuration to **8a** and **11a** and the C(9)α configuration to **10a**.

With the study of jatrophone complete, we turned our attention toward the other six derivatives (**2–7**). On the basis of the results observed for jatrophone (**1**), a generalized procedure was developed. First, each derivative was treated with propanethiol for 30 min, during which time the starting material was usually consumed. The ratio and types of products were then determined. Second, monoadducts, if formed, were resubmitted to the same conditions until no further reaction took place.

In the case of 2α-hydroxyjatrophone (**2**) such treatment produced only a trace of the bis(propanethiol) adduct **11b**, the major product being monoadduct **8b**. Bisadduct **11b** exhibited a NMR spectrum similar to that of **11a**, with the differences attributable to the presence of the C(2) hydroxyl group. For example, a single olefinic resonance at δ 5.45 was observed and the signal attributed to C(3)H (δ 3.59) showed only allylic coupling (2.5 Hz). Furthermore, the latter was shifted downfield by 1 ppm relative to the corresponding resonance in **11a**. Although the stereochemistry at C(3) could not be determined from coupling constants as in jatrophone, an NOE experiment indicated that the *n*-propylthio group was again α. Here, irradiation of the C(16) methyl group elicited a 13% enhancement of the C(3) methine proton. This result requires a cis vicinal relationship of the C(16) methyl and the C(3) proton. Finally, monoadduct **8b** was shown to possess the assigned structure and not that of **10b** by its further treatment with propanethiol whereupon it was converted completely to the bisadduct **11b** over the course of 4 h.

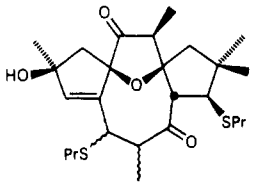
Like jatrophone (**1**), 2β-hydroxyjatrophone (**3**) produced three adducts after a 30-min exposure to propanethiol. In this case however the major product was **10c** and not **8c**. The ratio of **8c**, **10c**, and **11c** was 1:5:1. Again the NMR spectra of these products corresponded closely to the analogous products produced from **1** and **2**. When the mixture of monoadducts was subjected to further thiol treatment, the minor adduct **8c** was converted to **11c**, while the major adduct **10c** remained unchanged.

In addition to the chemical evidence for assigning the structures **8** and **10** to the monoadducts, the ^1H chemical shifts for the C(3) and C(5) olefinic resonances were consistent among the three sets of monoadducts. In **8a** and **8c**, the C(3,5) proton resonances were downfield of the olefinic protons observed in **10a** and **10c**. While only a single monoadduct was observed for **2**, the chemical shifts for the olefinic protons in **8b** were similar to those observed in **8a** and **8c**.

The final derivative to be examined that possessed the jatrophone skeleton was the synthetic derivative 2-normethyljatrophone (**4**). Initial treatment with propanethiol produced a mixture of the monoadducts **8d** and **10d** in a ratio of 4:1. The assignment of **8d** to the major adduct was made by comparison of the NMR spectrum of the mixture to the spectra of **8a** and **10a**. When the mixture was resubmitted to the reaction conditions, a complex mixture of products resulted. None of these when examined by NMR was found to be consistent with the expected bisadduct **11d**.

Turning next to 2β-hydroxy-5,6-isojatrophone (**5**), treatment with propanethiol produced one major and one minor product after 30 min. Both adducts exhibited chemical ionization mass spectra consistent with a bis(propanethiol) adduct. The major product obtained in 49% yield was assigned structure **12**. The presence of two methyl doublets in the NMR spectrum as well as the similarity of chemical shift and couplings for the C(8,9) protons with respect to the corresponding resonances in **10a–c** indicated that transannular bond formation between C(8) and C(12) had occurred with the indicated stereochemistry. That a second transannular bond had not formed was supported both by the IR spectrum, which revealed two carbonyl absorptions (1750 and 1690 cm^{-1}), and by the presence of a single olefinic resonance in the NMR at low field (δ 6.47). A third methyl doublet at δ 1.33 indicated that addition had occurred at C(5) and not at C(3). The NMR spectrum of the minor adduct also exhibited an olefinic resonance at low field (δ 6.33), but due to the small quantity of material the full spectrum could not be analyzed in detail.

Turning next to the jatropholactones, the trans isomer (**6**) readily formed an adduct (**13**) with propanethiol. A methyl singlet in the NMR spectrum at δ 1.84 and a single olefinic resonance at δ 6.28 indicated that addition had occurred at C(8) but without transannular bond formation.



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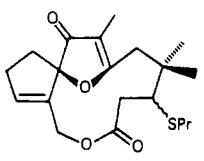

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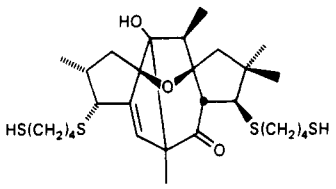

(14)

In addition, the IR spectrum exhibited a carbonyl absorption at 1710 cm^{-1} characteristic of the unsaturated 3(2*H*)-furanone system.[15]

The second synthetic analogue, *cis*-jatropholactone (**7**), was found to be inert to addition of propanethiol. Even after prolonged reaction (48 h) the starting lactone was recovered unchanged with no other products observed.

Finally, a previous report indicated that jatrophone (**1**) reacts with both thiol groups of a dithiol (e.g., dithiothreitol).[3a] In our hands, however, treatment of jatrophone with excess 1,4-butanedithiol produced only the bis(butanedithiol) adduct **13**. The stoichiometric reaction was not examined.

Discussion

In each case where conformation was determined in both the solid state and in solution (**1** and **4–6**), no significant differences were observed. The jatrophone derivatives **1–4** had essentially identical conformations in solution. Yet the relative amounts of the three adducts differed significantly among the four cases. Most remarkable was the effect of the change in hydroxyl stereochemistry at C(2). In 2α-hydroxyjatrophone (**2**), the only observed product was **8b** whereas for 2β-hydroxyjatrophone (**3**) the predominant product was **10c**. In a concerted conjugate addition–transannular bond forming process,[6b] **8** would result from a transoid conformation of the C(7) carbonyl and the C(8,9) olefin and **10** would result from a cisoid conformation. The ground-state conformations of **1–4** however have been shown to be transoid in each case.[8] Thus it appears that the conformation, at least in the ground state, is not a controlling factor in the reaction. In the addition to isojatrophone **5**, a transannular bond is also formed though clearly no π-orbital overlap between C(8) and C(12) is possible in the ground state or even after addition at C(9). It thus seems likely that addition first occurs at C(5), allowing sufficient flexibility in the macrocyclic ring for the necessary overlap to then take place.

These results suggest that the addition–transannular bond formation process occurs via a nonconcerted mechanism. The latter is supported by the results obtained for the synthetic analogues of jatrophone. For example, in the case of *trans*-jatropholactone (**6**) one might have predicted a product analogous to **10** relying on the observed cisoid conformation in the ground state (vide supra). That only simple conjugate addition took place suggests that less obvious factors are influencing the course of the reaction. Only in the case of *cis*-jatropholactone (**7**) is the situation clear. No addition takes place due to the lack of conjugation of the macrolide double bond and carbonyl. This result is also significant in that of the six derivatives only the noncytotoxic *cis*-jatropholactone (**7**) failed to undergo thiol addition.

Finally, the observation that the principle product of thiol addition to jatrophone (**1**), when allowed to go to completion, is a bis addition product is, we believe, of significance in terms of the chemical mechanism of the cytotoxic effect. That is, cross-linking of proteins by jatrophone may be the principle mode of cell inactivation.

Experimental Section

Materials and Methods. Commercial silica gel plates (250 μm) with a fluorescent indicator (E. M. Merck) were used for both analytical and preparative thin-layer chromatography. Visualization was accomplished by UV light and/or straining with 4% ethanolic 12-molybdophosphoric acid (Alfa). Infrared spectra were obtained for chloroform solutions on a Perkin-Elmer Model 337 spectrophotometer and ^1H NMR spectra were obtained for deuteriochloroform solutions at 250 MHz on a Burker WM-250 spectrometer, or where indicated an IBM WM-200 spectrometer. The internal standard was tetramethylsilane. High-resolution mass spectra were obtained by the University of Pennsylvania Mass spectrometry Service Center on a VG micromass 70/70H high-resolution double-focusing electron impact–chemical ionization spectrometer using isobutane as the reagent gas and interfaced with a Kratos DS-50-S data system. The borate buffer was prepared by treating a solution of boric acid with aqueous sodium hydroxide (1 N) to pH 9.2 on pH meter and then diluting to 0.1 M.

Reaction of Jatrophone (1) with *n*-Propanethiol. A solution of jatrophone (**1**, 8.8 mg, 28 μmol) in 0.5 mL of tetrahydrofuran was treated with 0.3 mL of 0.1 M, pH 9.2 borate buffer and 0.1 mL of propanethiol. After being stirred at room temperature for 30 min, the mixture was then poured into 5 mL of brine and extracted with ether. Preparative thin-layer chromatography (5:1 hexane–ethyl acetate) produced three UV active bands which were eluted with ethyl acetate. The fastest band after elution produced 6.3 mg (58%) of a 4:1 mixture of **8a** and **10a**; respectively (see the following for spectral data). The second band produced 1.3 mg (10%) of **11a** as a colorless film: IR 3600 (w), 3650–3200 (br), 2975 (s), 1705 (s), 1460 (m), 1215 (s) cm^{-1}; NMR δ 5.32 (s, 1 H), 3.77 (d, J = 12.4 Hz, 1 H), 3.11 (d, 5 = 7.7 Hz, 1 H), 2.95–2.70 (m, 2 H), 2.57 (br, 1 H, OH), 2.55 (t, J = 7.6 Hz, 2 H), 2.33 (d, J = 12.4 Hz, 1 H), 1.99 (q, J = 7.5 Hz, 1 H), 1.90 (d, J = 14.3 Hz, 1 H), 1.79 (q, J = 6.8 Hz, 1 H), 1.73 (d, J = 14.3 Hz, 1 H), 1.58 (m, 4 H), 1.46 (dd, J = 13.1, 10.6 Hz, 1 H), 1.30 (s, 3 H), 1.28 (m, 1 H), 1.13 (d, J = 6.8 Hz, 3 H), 1.08 (s, 3 H), 1.05 (s, 3 H), 1.00 (t, J = 7.5 Hz, 3 H), 0.96 (t, J = 7.8 Hz, 3 H), 0.83 (d, J = 7.5 Hz, 3 H); chemical-ionization mass spectrum, m/z 464.2376 (M), calcd for $C_{26}H_{40}O_3S_2$ 464.2418. The slowest band produced 0.9 mg (10%) of jatrophone (**1**).

Further Reaction of the Monothiol Adducts 8a and 10a. The mixture of the monothiol adducts (3.0 mg) obtained above (ca. 4:1 ratio of **8a** to **10a**) was subjected to the identical reaction conditions for an additional 1.5 h. Preparative thin-layer chromatography (6:1 hexane–ethyl acetate) produced two UV active bands. The fastest band, after elution with ethyl acetate, produced 0.5 mg (17%) of **10a** as a colorless film, which corresponded to the minor component of the starting mixture: IR 3600–2250 (br), 2950 (s), 2910 (s), 2910 (s), 1720 (s), 1650 (s), 1500 (m) cm^{-1}; NMR δ 6.11 (br s, 1 H), 6.04 (d, J = 2.9 Hz, 1 H), 3.60 (d, J = 12.8 Hz, 1 H), 2.88 (br, 1 H), 2.86 (d, J = 12.8 Hz, 1 H), 2.67 (q, J = 6.8 Hz, 1 H), 2.6–2.4 (m, 4 H), 2.32 (dd, J = 14.0, 7.8 Hz, 1 H), 1.98 (s, 3 H), 1.95 (m, 2 H), 1.75 (dd, J = 14.0, 2.1 Hz, 1 H), 1.28 (s, 3 H), 1.26 (s, 3 H), 1.20 (d, J = 6.8 Hz, 3 H), 1.07 (d, J = 6.8 Hz, 3 H), 0.86 (t, J = 7.4 Hz, 3 H); chemical-ionization mass spectrum, m/z 388.2069 (M), calcd for $C_{23}H_{32}O_3S$ 388.2072. Electronic subtraction of the NMR spectrum of **10a** from that of the mixture produced a difference spectrum attributed to **8a**: δ 6.60 (s, 1 H), 6.33 (s, 1 H), 3.76 (d, J = 13.1 Hz, 1 H), 3.12 (br q, J = 7.4 Hz, 1 H), 3.05 (d, J = 13.1 Hz, 1 H), 2.56 (m, 1 H), 2.47 (t, J = 7.5

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Hz, 2 H), 2.30 (dd, J = 13.1, 6.6 Hz, 1 H), 2.08 (s, 2 H), 1.88 (s, 3 H), 1.84 (dd, J = 13.1, 7.5 Hz, 1 H), 2 H unobserved between 1.65 and 1.20, 1.14 (s, 3 H), 1.11 (s, 3 H), 1.11 (d, J = 7.2 Hz, 3 H), 1.07 (d, J = 7.4 Hz, 3 H), 0.94 (t, J = 7.5 Hz, 3 H). The slow-moving UV active band produced 2.4 mg (67%) of **11a**, identical with the material previously isolated.

Reaction of 2α-Hydroxyjatrophone (2) with *n*-Propanethiol. A solution of 12.2 mg of **2** in 0.4 mL of tetrahydrofuran was treated with 0.3 mL of pH 9.2 buffer and 0.1 mL of propanethiol. After 30 min, the mixture was poured into brine and extracted with ether. Concentration and preparative thin-layer chromatography (2:1 hexane–ethyl acetate) of the residue produced two weakly UV active bands. The first band produced a trace of the bis(propanethiol) adduct **11b** (see spectroscopic data below). the second band after elution with ethyl acetate produced 11.4 mg (73%) of a colorless film corresponding to **8b**: IR 3580 (m), 3600–3200 (br m), 2995 (m), 2950 (s), 2920 (s), 2850 (m), 1745 (s), 1660 (s), 1220 (s) cm^{-1}; NMR δ 6.44 (br s, 1 H), 6.23 (s, 1 H), 3.79 (d, J = 13.8 Hz, 1 H), 3.04 (d, J = 13.8 Hz, 1 H), 2.54 (complex m, 3 H), 2.28 (s, 2 H), 2.18 (m, d, J = 14.5 Hz, 2 H), 1.92 (s, 3 H), 1.54 (m, ca. 2 H, partially obscured by water peak), 1.52 (s, 3 H), 1.17 (s, 3 H), 1.15 (d, J = 7.2 Hz, 3 H), 0.96 (t, J = 7.8 Hz, 3 H); chemical-ionization mass spectrum, m/z 404.2023 (M), calcd for $C_{23}H_{32}O_4S$ 404.2021.

A solution of **8b** (6.1 mg) was further treated with propanethiol under the identical conditions. Thin-layer chromatography showed **8b** to be gradually consumed over 4.5 h. Usual workup and preparative thin-layer chromatography (2:1 hexane–ethyl acetate) produced the bisadduct **11b** as the sole product as a white film (5.1 mg, 70%): IR 3600–3200 (br, m), 3020 (m), 2960 (s), 1705 (s), 1605 (s), 1215 (s) cm^{-1}; NMR δ 5.45 (d, J = 2.5 Hz, 1 H), 3.68 (d, J = 12.8 Hz, 1 H), 3.59 (d, J = 2.5 Hz, 1 H), 3.10 (s, 1 H), 2.96–2.70 (complex m, 3 H), 2.66 (t, J = 7.2 Hz, 2 H), 2.42 (d, J = 15 Hz, 1 H), 2.34 (d, J = 2.5 Hz, 1 H), 2.32 (d, J = 11.0 Hz, 1 H), 1.96 (q, J = 7.0 Hz, 1 H), 1.83 (d, J = 14.4 Hz, 1 H), 1.78 (d, J = 14.4 Hz, 1 H), 1.78–1.52 (complex m, 4 H), 1.24 (s, 3 H), 1.08 (s, 3 H), 1.04 (s, 3 H), 1.02 (t, J = 7.5 Hz, 3 H), 0.98 (t, J = 7.8 Hz, 3 H), 0.83 (d, J = 7.0 Hz, 3 H). Chemical-ionization mass spectrum, m/z 480.2327 (M), calcd for $C_{26}H_{40}O_4S_2$ 480.2368.

Reaction of 2β-Hydroxyjatrophone (3) with *n*-Propanethiol. A solution of 6.8 mg of **3** in 0.4 mL of tetrahydrofuran was treated with 0.3 mL of buffer and 0.1 mL of propanethiol. After 30 min, the reaction mixture was worked up in the usual way. Preparative thin-layer chromatography (2:1 hexane–ethyl acetate) produced 8.1 mg of a colorless film. The NMR spectrum of the mixture indicated it to contain **8c**, **10c**, and **11c** in a ratio of 1:5:1. A second preparative chromatography separated **11c** from the monoadducts (see below for spectral data).

The mixture of monoadducts (6.2 mg) was treated again with propanethiol under the same conditions. After 6 h, the mixture was worked up in the usual manner. Preparative thin-layer chromatography recovered the major monoadduct **10c** (4.3 mg, 69%) and the bisadduct **11c** (0.9 mg, 12%).

10c: IR 3580 (m), 3600–3300 (br m), 2960 (s), 2920 (s,), 2860 (m), 1740 (s), 1660 (s), 1220 (s) cm^{-1}; NMR δ 6.07 (s, 1 H), 5.99 (s, 1 H), 3.61 (d, J = 13.4 Hz, 1 H), 2.84 (dm, J = 13.4 Hz, 1 H), 2.68 (q, J = 7.0 Hz, 1 H), 2.55 (complex m, 2 H), 2.33 (d, J = 13.8 Hz, 1 H), 2.21 (d, J = 13.8 Hz, 1 H), 2.09 (s, 2 H), 1.98 (s, 3 H), 1.72 (br, 1 H), 1.56 (m, ca. 2 H, partially obscured by water peak), 1.45 (s, 3 H), 1.20 (d, J = 7.0 Hz, 3 H), 1.10 (s, 3 H), 0.97 (t, J = 7.2 Hz, 3 H). Chemical-ionization mass spectrum, m/z 404.2032 (M), calcd for $C_{23}H_{32}O_4S$ 404.2021.

11c: IR 3600–3200 (br m), 3020 (m), 2960 (s), 2930 (s), 2870 (m), 1705 (s), 1650 (s), 1230 (s). NMR δ 5.39 (s, J = 1.6 Hz, 1 H), 3.78 (d, J = 12.2 Hz, 1 H), 3.55 (d, J = 1.6 Hz, 1 H), 3.52 (s, 1 H), 2.96–2.70 (complex m, 2 H), 2.60 (t, J = 7.5 Hz, 2 H), 2.32 (d, J = 12.2 Hz, 1 H), 2.05–1.83 (m, 3 H), 1.98 (d, J = 14.4 Hz, 1 H), 1.92 (d, J = 14.4 Hz, 1 H), 1.78–1.67 (m, 3 H), 1.60 (m, ca. 2 H, partially obscured by water peak), 1.38 (s, 3 H), 1.31 (s, 3 H), 1.08 (s, 3 H), 1.01 (t, J = 7.6 Hz, 3 H), 0.99 (t, J = 7.5 Hz, 3 H), 0.84 (d, J = 7.2 Hz, 3 H). Chemical-ionization mass spectrum, m/z 480.2358 (M), calcd for $C_{26}H_{40}O_4S_2$ 480.2368.

8c: partial NMR from difference spectrum δ 6.50 (s, 1 H), 6.26 (s, 1 H), 3.83 (d, J = 13.3 Hz, 1 H), 3.03 (d, J = 13.3 Hz, 1 H).

Reaction of 2β-Hydroxy-5,6-isojatrophone (5) with *n*-Propanethiol. A solution of 6.6 mg of 2β-hydroxy-5,6-isojatrophone (25.7 μmol) in 0.3 mL of THF was treated with 0.2 mL of pH 9.2 borate buffer and 0.1 mL of propanethiol. After 30 min, thin-layer chromatography showed one minor and two major spots all weakly UV active and faster moving than the starting material, which was no longer observed. The mixture was poured into brine, extracted with ether, concentrated, and the residue purified by preparative thin-layer chromatography (2:1 hexane–ethyl acetate). The second band produced, after elution with ethyl acetate, 6.1 mg (49%) of **12** as a colorless film: IR 3650–3400 (br m), 3025 (m), 2970 (s), 2930 (s), 2870 (m), 1750 (s), 1690 (s), 1460 (m), 1220 (s) cm^{-1}; NMR δ 6.47 (d, J = 1.4 Hz, 1 H), 3.67 (d, J = 14.4 Hz, 1 H), 3.32 (dd, J = 12.2, 1.4 Hz, 1 H), 3.23 (m, 1 H), 2.87 (d, J = 14.4 Hz, 1 H), 2.65–2.43 (complex m, 5 H), 2.29 (d, J = 13.9 Hz, 1 H), 2.13 (d, J = 13.9 Hz, 1 H), 2.11 (d, J = 14.9 Hz, 1 H), 2.02 (d, J = 14.9 Hz, 1 H), 1.80 (br s, 1 H), 1.58 (m, J = 7.6 Hz, ca. 4 H, partially obscured by water peak), 1.39 (s, 3 H), 1.33 (d, J = 6.8 Hz, 3 H), 1.27 (d, J = 6.3 Hz, 3 H), 1.25 (br s, 3 H), 1.06 (s, 3 H), 1.02 (t, J =7.1 Hz, 3 H), 0.98 (t, J = 7.6 Hz, 3 H); chemical-ionizaton mass spectrum, m/z 480.2380 (M^+), calcd for $C_{26}H_{40}O_4S_2$ 480.2368. From the fastest UV active band, after a second chromatography (3:1 ether–hexane, two developments), was isolated a trace of a second bis(*n*-propanethiol) adduct, which could not be completely characterized: NMR (partial) δ 6.22 (s, 1 H), 3.63 (d, J = 8.4 Hz, 1 H), 3.50 (d, J = 3.8 Hz, 1 H), 3.38 (d, J = 8.4 Hz, 1 H). Chemical-ionization mass spectrum, m/z 480.2380 (M) calcd for $C_{26}H_{40}O_4S_2$ 480.2368.

Reaction of *trans*-Jatropholactone (6) and *n*-Propanethiol. A solution of 7.0 mg of **6** in 0.4 mL of tetrahydrofuran was treated with 0.3 mL of pH 9.2 borate buffer and 0.1 mL of propanethiol and stirred in a stoppered flask until thin-layer chromatography showed **6** to be completely consumed (ca. 30 min). A single UV-active spot was observed. Preparative thin-layer chromatography (3:1 hexane–ethyl acetate) produced 6.2 mg (71%) of a colorless oil: IR 2950 (s), 1710 (s), 1680 (s), 1590 (s), 1210 (m) cm^{-1}; NMR δ 6.28 (br d, J = 2.0 Hz, 1 H), 5.26 (d, 13.1 Hz, 1 H), 4.29 (apparent dq, J = 13.1, 2.0 Hz, 1 H), 3.28 (dd, J = 6.9, 4.1 Hz, 1 H), 2.90 (dd, J = 17.5, 6.2 Hz, 1 H), 2.85 (d, J = 14.4 Hz, 1 H), 2.66, 2.64 (m, dd, J = 17.5, 4.1 Hz, 2 H total), 2.48, 2.45 (m, t, J = 7.6 Hz, 4 H total), 2.33, 2.32 (ddd, J = 13.8, 5.6, 2.3 Hz; d, J = 14.4 Hz, 2 H total), 2.06 (ddd, J = 13.8, 7.5, 2.3 Hz, 1 H), 1.84 (s, 3 H), 1.58 (m, ca. 2 H, partially obscured by water peak), 1.35 (s, 3 H), 1.16 (s, 3 H), 0.96 (t, J = 7.5 Hz, 3 H); chemical-ionization mass spectrum, m/z 364.1716 (M), calcd for $C_{20}H_{28}O_4S$ 364.1708.

Reaction of Jatrophone (1) with 1,4-Butanedithiol. A mixture of jatrophone (5 mg), 1,4-butanedithiol (0.1 mL), and 0.3 mL of pH 9.2 borate buffer in 0.5 mL of tetrahydrofuran was stirred at room temperature overnight (ca. 16 h) in a stoppered flask. The mixture was then poured into brine and extracted with ether. Preparative thin-layer chromatography (5:1 hexane–ethyl acetate) produced a single weakly UV-active band, which was eluted with ethyl acetate and concentrated to yield a colorless oil, 9 mg (100%): IR 3600 (w), 3650–2250 (br), 2965 (s), 2935 (s), 1710 (s), 1450 (m), 1220 (s) cm^{-1}; NMR δ 5.32 (s, 1 H), 3.78 (d, J = 12.2 Hz, 1 H), 3.76 (br, 1 H) 3.13 (d, J = 7.2 Hz, 1 H), 2.88 (m, 2 H), 2.56 (m, 8 H), 2.34 (d, J = 12.2 Hz, 1 H), 2.00 (q, J = 6.8 Hz, 1 H), 1.9–1.6 (complex multiplets, 11 H), 1.49 (dd, J = 13.6, 10.8, 1 H), 1.35 (m, 2 H), 1.30 (s, 3 H), 1.12 (d, J = 6.8 Hz, 3 H), 1.10 (s, 3 H), 1.07 (s, 3 H), 0.83 (d, J = 7.7 Hz, 3 H); chemical-ionization mass spectrum, m/z 557.2207 (M + 1), calcd for $C_{28}H_{45}O_3S_4$ 557.2251.

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