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Carbon-13 Magnetic Resonance of Cotton Terpenoids: Carbon–Proton Long-Range Couplings

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The ^{13}C NMR spectra of five sesquiterpenoids and three C_{25} terpenoids found in *Gossypium hirsutum* and *G. Barbadense* cottons have been analyzed. Chemical shift assignments for the aryl and carbonyl carbons were made using long-range carbon–proton couplings and single frequency irradiation before and after deuterium exchange. Large deuterium isotope shifts are observed for the exchange of the hydroxy proton that is syn and strongly hydrogen bonded to the aldehyde function.

Some primitive varieties of cotton are more resistant than cultivated cottons toward the cotton bollworm and tobacco budworm (*Heliothis* spp.).[1] This greater resistance has been correlated with higher concentrations of several terpenoids, such as hemigossypolone (**1a**) and its 7-methyl ether derivative (**1b**), and the C_{25} terpenoids, such as heliocides H_1 (**3a**), B_1 (**3b**), and H_2 (**3c**).[2,3] The structures of hemigossypolone and **3c** were determined by mass, proton, and carbon-13 spectra. Compound **3c** was synthesized from hemigossypolone and its stereochemistry determined by x-ray crystal analysis.[4,5] The structures of **3a** and **3b** were based upon syntheses and carbon-13 spectra.[6–8] The carbon-13 spectra were essential in assigning the structures of several of the heliocides. However, it was not possible to make unambiguous shift assignments for carbons 2, 6, 7, or 8 or to distinguish between the bridgehead carbons 9 and 10 or the carbonyl carbons 1 and 4 with proton-decoupled spectra. It was also important to study the carbon-13 spectra of the sesquiterpenoids hemigossypol (**2a**), methoxyhemigossypol (**2b**) (the biosynthetic precursors of **1a** and **1b**[9]), and gossypol (**2c**) because of their biosynthetic relationship to the heliocides and to assist in the analysis of new, structurally related terpenoids.

One-bond couplings and off-resonance decoupling are of little value in uniquely assigning the aromatic and carbonyl carbons because most are quaternary. Shift assignments based upon additivity relationships also fail because of the many ortho interactions.[10] However, proton-decoupled carbon-13 chemical shifts are useful in the structural analysis of polysubstituted aromatic natural products once shift assignment ambiguities are resolved, and chemical shift changes are correlated with substituent changes.[6,7] This is especially important when the quantity isolated from natural sources precludes the use of coupled spectra as an assignment technique.[6]

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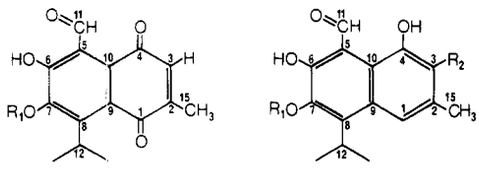
Table I. Carbons 2, 3, and 5: Chemical Shifts and Proton-Carbon Couplings[a]

Compd	Registry no.	C-2 Shift, δ	C-2 $^2J_{H15}$	C-3 Shift, δ	C-3 $^1J_{H3}$	C-3 $^3J_{H1}$	C-3 $^3J_{H15}$	C-5 Shift, δ	C-5 $^2J_{H11}$	C-5 $^3J_{OH6}$
1a	35688-47-2	147.9	6.7	132.4	166.5		5.9	114.9	20.3	*b*
b	35839-49-7	149.3	6.3	133.4	166.5		5.4	117.0	19.6	4.2
2a	40817-07-0	132.8	6.0	111.7	156.1	7.3	4.8	110.9	17.8	*b*
b	50399-95-6	132.7	6.0	113.1	156.2	7.5	4.7	112.4	18.2	*c*
c	303-45-7	132.9	5.5	116.8		8.4	4.2	111.1	17.9	*b*
3a	64872-64-6	49.0	*d*	57.3	*d*	*d*	*d*	114.1	20.0	*b*
b	64872-35-1	49.0	*d*	56.9	*d*	*d*	*d*	115.3	19.8	5.2
c	63525-06-4	49.3	*d*	54.8	*d*	*d*	*d*	115.2	19.8	*b*

[a] Chemical shifts are in ppm downfield from Me_4Si using central resonance of $CDCl_3$ as an internal reference; solvent for **1b, 3a, 3b,** and **3c** was deuteriochloroform and solvent for **1a, 2a, 2b,** and **2c** was acetone; coupling constants are in Hz (±0.5 Hz); u, unresolved multiplet. [b] Coupling to C-6 hydroxy proton not observed. [c] Coupling to C-6 hydroxy proton not observed because coupled spectrum was obtained in acetone. [d] Not determined.

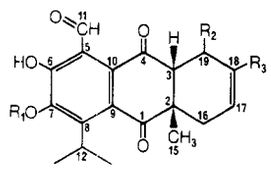


1

a: R_1 = H
b: R_1 = CH_3

2

a: R_1, R_2 = H
b: R_1 = CH_3, R_2 = H
c: R_1 = H, R_2 = ⟩$_2$



3

a: R_1 = H, R_2 = $CH_2CH=C(CH_3)_2$, R_3 = CH_3
b: R_1 = CH_3, R_2 = $CH_2CH=C(CH_3)_2$, R_3 = CH_3
c: R_1, R_2 = H, R_3 = $CH_2CH_2CH=C(CH_3)_2$

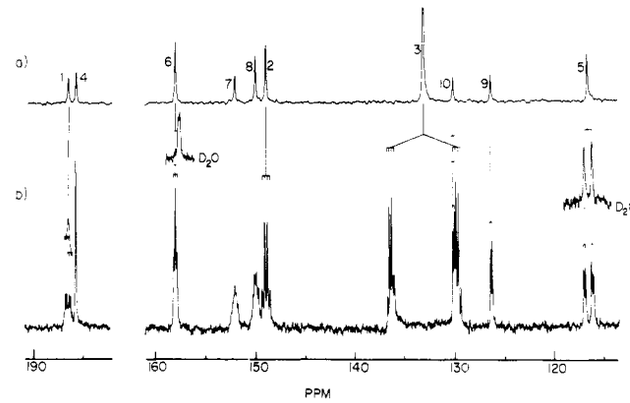


Figure 1. Carbon-13 spectra of the carbonyl and aryl regions of 7-methoxyhemigossypolone (**1b**): (a) proton-decoupled spectrum before deuterium exchange; (b) proton-coupled spectrum before and after deuterium exchange. Upfield change in carbon 6 after deuterium exchange is due to deuterium isotope shift.

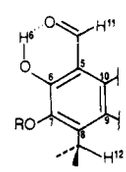
Recent reports for phenols,[11] courmarins,[12,13] flavanones, and flavones[14] have shown the usefulness of long-range proton–carbon couplings in the assignment of quaternary carbon resonances. We report here a detailed analysis of the long-range couplings for hemigossypolone (**1a**), hemigossypolone 7-methyl ether (**1b**), hemigossypol (**2a**), 7-methoxyhemigossypol (**2b**), and gossypol (**2c**). Also, the naturally occuring C_{25} terpenoids **3a** (derived from ocimene and hemigossypolone), **3b** (derived from ocimene and 7-methoxyhemigossypolone), and **3c** (derived from myrcene and hemigossypolone) have been studied. Analysis of the long-range couplings before and after exchange with deuterium oxide combined with single frequency irradiation of various hydrogens allows unequivocal assignments for all quaternary carbons. Further, these long-range coupling patterns give insight into stereochemical features of these molecules in solution.

Results and Discussion

Chemical Shift Assignments for Carbons 2, 3, and 5. In all the sesquiterpenoids, carbon 2 is easily assigned in the proton-coupled spectra because it is a well-resolved quartet due to two-bond coupling to the C-15 methyl hydrogens. In the C_{25} terpenoids, carbon 2 is the only quaternary carbon in the alkyl region (Table I).

Carbon 3 for **1a** and **1b** shows a large one-bond coupling to the directly attached hydrogen and a three-bond coupling to the C-15 methyl hydrogens and is therefore also easily assigned. In **2a** and **2b,** two one-bond doublets are present because of hydrogens at carbons 1 and 3. Carbon 3 is assigned to the upfield doublet because greater shielding is experienced by the carbon ortho to the C-4 hydroxy. For gossypol (**2c**), carbon 3 is a quaternary carbon at δ 116.8. For **3a, 3b,** and **3c,** carbon 3 is a doublet in the alkyl region.

All the compounds displayed an upfield aromatic resonance between δ 110 and 117, assigned to carbon 5 because of the large two-bond coupling to H-11. The shielding of these resonances compared to carbon 1 of benzaldehyde (δ 137.7) is due to intramolecular hydrogen bonding of the aldehyde. A further splitting of the C-5 resonance is observed for the 7-methoxy compounds, **1b** and **3b,** when dissolved in deuteriochloroform. Figure 1 presents the decoupled and coupled spectra of the aryl and carbonyl regions for **1b** which shows this further coupling at carbon 5. The smaller coupling disappears upon dueterium exchange. Since **1b** and **3b** have only one exchangeable proton (R_1 = CH_3), this fine structure must be due to three-bond coupling between the C-6 hydroxy proton and carbon 5 (**4**). The magnitude of this coupling indicates that



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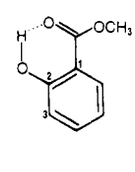

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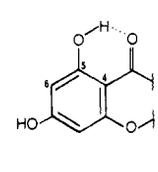


6

the stereochemistry of the C-6 hydroxy function is syn to the aldehyde and strongly hydrogen bonded to it.[15] Similar syn couplings have been observed between the hydroxy proton and C-1 in methyl salicylate (**5**, $^3J_{C1-OH^2}$ = 4.4 Hz)[11] and between the 5-hydroxy proton and C-4 in 5-hydroxyflavones and

Table II. Carbons 6, 7, and 8: Chemical Shifts and Proton–Carbon Couplings[a]

Compd	C-6 Shift, δ	C-6 $^2J_{OH^6}$	C-6 $^3J_{H^{11}}$	C-7 Shift, δ	C-7 $^3J_{H^{12}}$	C-7 $^4J_{H^{11}}$	C-8 Shift, δ
1a	151.3	*b*	4.2	148.5	6.7	2.7	139.9
b	158.4	4.4	4.4	152.4	u	2.5	150.4
2a	154.7	*b*	5.0	142.0	5.8	2.9	133.2
b	159.1	*c*	4.4	145.5	u	u	143.3
c	155.0	*b*	4.2	142.5	6.0	2.9	132.9
3a	152.5	*b*	4.1	148.3	5.9	2.4	140.3
b	158.8	4.2	4.2	151.9	u	u	149.7
c	152.2	*b*	3.9	148.9	~7	~2	140.0

[a] Chemical shifts are in ppm downfield from Me_4Si using central resonance of $CDCl_3$ as an internal reference; solvent for **1b, 3a, 3b,** and **3c** was deuteriochloroform and solvent for **1a, 2a, 2b,** and **2c** was acetone; coupling constants are in Hz (±0.5 Hz); u, unresolved multiplet. [b] Coupling to C-6 hydroxy proton not observed. [c] Coupling to C-6 hydroxy proton not observed because coupled spectrum was obtained in acetone.

5-hydroxyflavanones (**6**, $^3J_{C^4\text{-}OH^5}$ = 4.5 Hz).[14a] The anti three-bond coupling of a hydroxy proton is usually larger (e.g., **5**, $^3J_{C^3\text{-}OH^2}$ = 7.5 Hz).[11] The anti coupling to carbon 7 was not observed for **1b** or **3b** because carbon 7 is an unresolved multiplet due to coupling to other hydrogens.

None of the other compounds displayed this three-bond coupling of the C-6 hydroxy proton to carbon 5. For this coupling to be observed, intramolecular and intermolecular proton exchange must be slow on the carbon-13 time scale. For the compounds studied in deuteriochloroform (**3a** and **3c**), absence of this coupling implies an increase in the rate of proton exchange, perhaps because of the presence of two hydroxy functions. For compounds with low solubility in deuteriochloroform (**1a, 2a, 2b,** and **2c**), coupled spectra were obtained in acetone. In this more polar solvent, the rate of exchange is expected to be more rapid and coupling of carbon 5 to the C-6 hydroxy proton is lost.

Chemical Shift Assignments for Carbons 6, 7, and 8. The resonances due to the oxygen-substituted carbons 6 and 7 and the isopropyl-substituted carbon 8 were the most difficult to distinguish from one another. These resonances are expected between δ 130 and 160. The long-range couplings of carbons 6, 7, and 8 and the chemical shift assignments based upon these couplings are presented in Table II.

The coupled spectrum of **1b** shows four resonances between δ 149 and 159 (Figure 1b). The clearly resolved quartet at δ 149.3 has already been assigned to carbon 2. Carbon 6 may be distinguished from carbons 7 and 8 in **1b** and the other 7-methoxy compounds (**2b** and **3b**) because of long-range coupling to the hydroxy and aldehydic hydrogens. The resonance at δ 158.4 is a "triplet" which becomes a doublet and undergoes an isotope shift upon deuterium exchange. Taken together with the concurrent loss of coupling at carbon 5 upon deuterium exchange, the triplet must be due to two-bond coupling to OH-6 and three-bond coupling to H-11 of the same magnitude.

The two remaining downfield aryl resonances at δ 152.4 and 150.4 must be due to carbons 7 and 8. These unresolved multiplets can not be unequivocally assigned based upon their long-range couplings alone. Simultaneous irradiation of H-12 and the 7-methoxy hydrogens (^1H NMR δ 4.03 and 3.98, respectively) after deuterium exchange causes the downfield resonance to collapse to a doublet ($^4J_{C^7\text{-}H^{11}}$ = 2.5 Hz), while the upfield resonance remains a multiplet due to coupling to the isopropyl methyls. Therefore, the downfield resonance is assigned to carbon 7 and the upfield resonance to carbon 8.

Figure 2 shows the coupled, decoupled, and single frequency decoupled spectra for hemigossypolone (**1a**), typical of the coupling patterns observed after deuterium exchange for compounds with hydroxy groups at both carbons 6 and 7. In the coupled spectrum (Figure 2b), the furthest downfield aryl

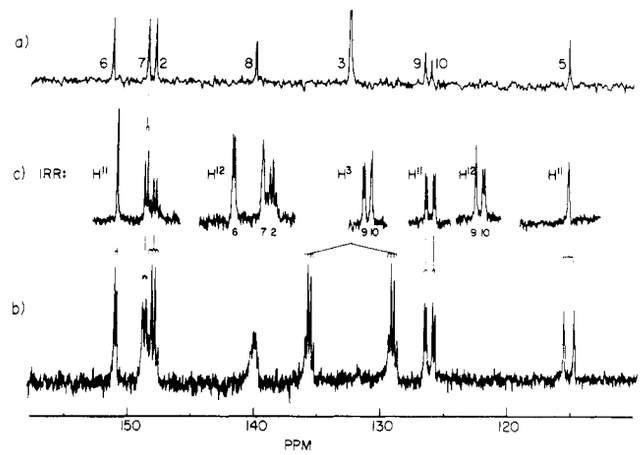
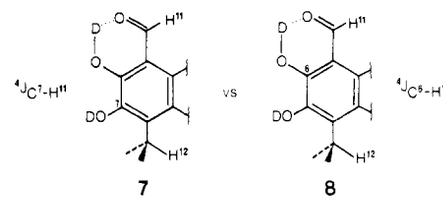


Figure 2. Carbon-13 spectra of the aryl region of hemigossypolone (**1a**): (a) proton-decoupled spectrum before deuterium exchange; (b) proton-coupled spectrum after deuterium exchange (upfield change in carbon 6 is due to deuterium isotope shift); (c) proton-coupled spectra with single frequency irradiation of various hydrogens.

resonance is a doublet due to coupling to H-11 and is therefore assigned to carbon 6. The sharp quartet at δ 147.9 is assigned to carbon 2. The resonance between carbons 2 and 6 is a doublet of doublets and is assigned to carbon 7. This coupling pattern is due to a larger three-bond coupling to H-12 and a smaller four-bond coupling to H-11 (**7**). Similar patterns were found for carbon 7 in the deuterium exchanged spectra of **2a,**



2c, and **3a** (Figures 3, 4, and 5). For **3c,** the four-bond coupling was not resolved and only a broadened doublet was observed.

It might be argued that the assignments of carbons 6 and 7 for **1a** and for the other compounds with hydroxy functions at 6 and 7 should be reversed. If this were the case, carbon 6 would be a doublet of doublets due to three-bond coupling to H-11 and four-bond coupling to H-12 (**8**). To eliminate this possibility, H-11 and H-12 were individually irradiated. Irradiation of H-11 removed the three-bond coupling to carbon 6, the two-bond coupling to carbon 5, and only the smaller four-bond coupling to the doublet of doublets assigned to carbon 7 (Figure 2c). Irradiation of the isopropyl hydrogen,

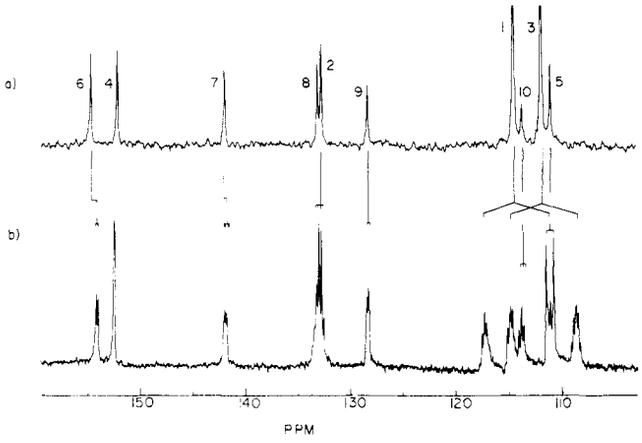


Figure 3. Carbon-13 spectra of the aryl region of hemigossypol (**2a**): (a) proton-decoupled spectrum before deuterium exchange; (b) proton-coupled spectrum after deuterium exchange. Upfield change in carbons 6 and 7 is due to deuterium isotope shift.

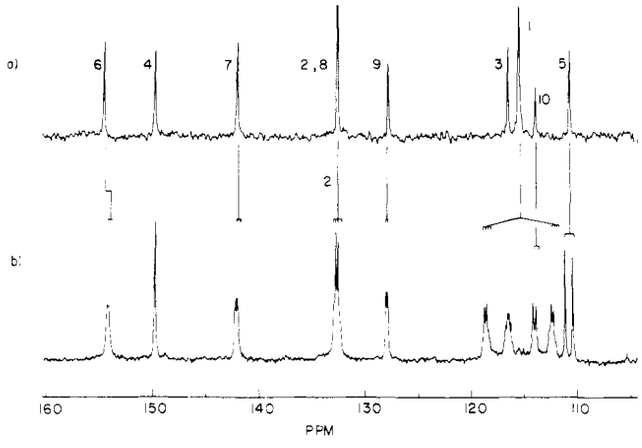


Figure 4. Carbon-13 spectra of the aryl region of gossypol (**2c**): (a) proton-decoupled spectrum before deuterium exchange; (b) proton-coupled spectrum after deuterium exchange. Upfield change in carbon 6 is due to deuterium isotope shift.

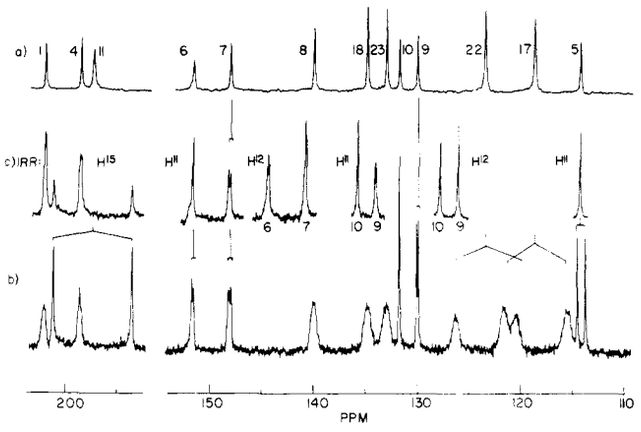


Figure 5. Carbon-13 spectra of the carbonyl, aryl, and alkenyl regions of heliocide H_1 (**3a**): (a) proton-decoupled spectrum after deuterium exchange; (b) proton-coupled spectrum after deuterium exchange; (c) proton-coupled spectra with single frequency irradiation of various hydrogens. Resonances numbered 17, 18, 22, and 23 are alkenyl carbons in the cyclohexenyl ring and in the R_2 side chain.

Table III. Deuterium Isotope Shifts[a]

Compd	C-6	C-7	C-5	C-8	Other
1a	−8.3	+4.3	+4.3	*b*	
b	−8.4	−1.5	*b*	*b*	
2a	−17.9	−5.6	*b*	*c*	+3.8 (C-4)
b	−19.6	−5.9	*b*	+2.4	+2.3 (C-4)
c	−17.9	−5.3	−4.0	−3.7	−7.5 (C-4)
3a	−12.6	−3.4	*b*	−2.3	
b	−8.9	−1.8	*b*	+1.9	
c	−10.7	−5.3	*b*	−4.1	
Salicyl-aldehyde[d]	−6.9	−3.9	*c*	−2.8	

[a] Shift changes are in Hz; negative values are upfield changes. [b] Shift change is within experimental error (±1.0 Hz). [c] Unobserved because of overlap with another resonance. [d] Salicylaldehyde was numbered to correspond to other compounds.

H-12, decoupled the larger three-bond coupling to carbon 7 and did not affect carbons 5 or 6.

The distinction between carbons 6 and 7 is confirmed by the isotope shifts that are observed upon deuterium exchange (Table III). The resonances assigned to carbon 6 consistently show a large shielding when deuterium replaces protium. Previously reported deuterium isotope shifts have been approximately 6 Hz for directly bonded deuterium and approximately 3 Hz for deuterium two bonds away.[16] Therefore, the shielding through two bonds at carbon 6 upon exchange is unusually large (8–20 Hz). These unusually large isotope shifts at carbon 6 are undoubtedly due to the exchange of a strongly hydrogen-bonded proton.[17] The resonances assigned to carbon 7 show smaller shieldings even in those cases where a proton on the C-7 hydroxy is being exchanged (**2a, 2c, 3a,** and **3c**). For **1a,** carbons 7 and 5 are deshielded upon exchange. The reason for this effect is unknown. At carbons further removed from the site(s) of exchange the effect is negligible.

It is important to note that carbon 7 in the methoxy compounds, **1b, 2b,** and **3b,** is consistently *upfield* from the C-6 hydroxy carbon (Table II). Simple additive shift relationships predict just the opposite, that the methoxy carbon should be *downfield* by approximately 6 ppm. However, this argument does not take into account the dominant effect which must account for the chemical shift relationship between these two oxygen-substituted vicinal carbons: the strong intramolecular hydrogen bonding between the C-6 hydroxy proton and the aldehyde oxygen (**4**). This influence is deshielding at carbon 6 if one compares phenol to salicylaldehyde.

The remaining downfield aryl resonance in the coupled spectrum of **1a** at δ 139.9 must be assigned to carbon 8 (Figure 2b). The shape of this resonance (a "broadened quartet") may be qualitatively analyzed as a doublet of septets where $^2J_{H12} > {}^3J_{H13}$. The resonance at δ 150.4 in the coupled spectrum of **1b** assigned to carbon 8 has this same broadened quartet shape (Figure 1b). Similarly shaped resonances in the coupled spectra of the other compounds allow the qualitative assignment of carbon 8.

Figures 3 and 4 present the decoupled and deuterium exchanged, coupled spectra for hemigossypol (**2a**) and gossypol (**2c**). These spectra are typical of the patterns observed for compounds containing two aromatic rings and an additional hydroxy substituent at carbon 4. This additional oxygen-substituted carbon appears as a sharp singlet after deuterium exchange and is not appreciably coupled to the hydrogens at carbon 1 and 3 or to the methyl group at carbon 2.

Chemical Shift Assignments for the Bridgehead Carbons 9 and 10. Carbons 9 and 10 are most easily distinguished in the compounds with two aromatic rings, **2a, 2b,** and **2c** (Figures 3 and 4, Table IV). In these compounds the principle chemical shift influence is the hydroxy group at carbon 4. This substituent is ortho and strongly shields carbon 10 compared to 9. Carbon 10 appears upfield as a triplet in the coupled

Table IV. Carbons 9 and 10: Chemical Shifts and Proton–Carbon Couplings[a]

Compd	C-9 Shift, δ	C-9 $^3J_{H12}$	C-10 Shift, δ	C-10 $^3J_{H3}$	C-10 $^3J_{H1}$
1a	126.4	4.1	125.9	4.9	
b	126.7	3.6	130.5	4.7	
2a	128.3	3.2	113.6	~6	~6
b	127.9	3.8	116.3	*b*	*b*
c	128.1	3.1	114.3		6.3
3a	130.0	4.2	131.8	<1	
b	129.0	4.5	135.9	<1	
c	131.5	4.5	129.1	<1	

[a] Chemical shifts are in ppm downfield from Me_4Si using central resonance of $CDCl_3$ as an internal reference; solvent for **1b, 3a, 3b,** and **3c** was deuteriochloroform and solvent for **1a, 2a, 2b,** and **2c** was acetone; coupling constants are in Hz (±0.5 Hz). [b] Overlap with C-3 resonance.

spectrum of hemigossypol (Figure 3b). This triplet is due to three-bond couplings to H-1 and H-3. In gossypol, this resonance becomes a doublet because of the absence of a hydrogen at carbon 3 (Figure 4b). It is important to note that carbon 10 is *not* coupled to the aldehydic hydrogen, H-11.

In the sesquiterpenoids **1a** and **1b** and in the C_{25} terpenoids **3a, 3b,** and **3c,** carbons 9 and 10 differ by 6 ppm or less and are more difficult to distinguish. For **1a,** irradiation of H-3 collapses the upfield doublet, and this resonance is therefore assigned to carbon 10 (Figure 2c). Irradiation of H-12 collapses the downfield doublet due to carbon 9. Similarly, carbon 10 is coupled to H-3 and carbon 9 to H-12 in **1b,** but their relative chemical shift positions are reversed, carbon 10 being downfield of carbon 9.

In the C_{25} terpenoids, carbon 10 is an uncoupled sharp singlet because the hydrogen at carbon 3 is now attached to an sp^3 rather than an sp^2 carbon. Carbon 9 is a doublet due to coupling to H-12 (Figure 5c). In all the compounds studied, irradiation of H-11 has no effect on the multiplicity of the resonances due to carbons 9 and 10. Thus, the aldehydic hydrogen is *not* appreciably coupled to either of these carbons.

It has been reported that three-bond carbon–proton couplings through sp^2 carbons are stereochemically dependent, with anti couplings consistently larger than syn couplings.[18] For example, in methyl salicylate the anti coupling of the hydroxy proton to carbon 3 is larger than the syn coupling to carbon 1 (**5,** 7.5 Hz vs. 4.4 Hz).[11] Similarly, the anti three-bond couplings of hydroxy protons in flavanoids and coumarins are 7–10 Hz while the corresponding syn couplings are 4–6 Hz.[12,14] In the present work, a similar stereochemical dependence has been found for the couplings of the aldehydic hydrogen to carbons 6 and 10. The anti couplings to carbon 6 range from 3.9 to 5.0 Hz (Table II, C-6), while no syn coupling is observed to carbon 10. This further confirms the syn stereochemistry of the aldehyde and the C-6 hydroxy function (**4**).[15]

Aryl Carbon Chemical Shift Changes. With the chemical shifts of all the aryl carbons firmly established through long-range couplings and deuterium exchange, it is worthwhile to compare the changes that occur in going from the 6,7-dihydroxy compounds (**1a, 2a,** and **3a**) to the corresponding 6-hydroxy-7-methoxy compounds (**1b, 2b,** and **3b**). Similar Δδ values are observed at all carbons for the three comparisons that may be made (Table V). The chemical shift changes observed at carbon 7 are close to the values predicted if one compares phenol to anisole. However, the carbons ortho and para to carbon 7 (carbons 6, 8, and 10) experience deshieldings upon change from hydroxy to methoxy. The reason for these deshieldings is not clear. They may be caused by steric interaction between the methoxy methyl and the isopropyl group in **1b, 2b,** and **3b.** This interaction may lead to a decrease in electron donation by the oxygen into the aryl ring and less shielding than expected, particularly at the para and ortho carbons, when compared to the shift changes observed in going from phenol to anisole. Whatever the exact explanation, it is important to be aware of these deshieldings because the change from hydroxy to methoxy is a frequent variation in structure in the terpenoids found in cotton.

Table V. Aryl Carbon Chemical Shift Changes: 7-Hydroxy → 7-Methoxy[a]

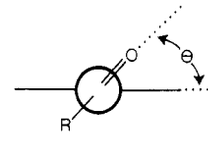
	C-5	C-6	C-7	C-8	C-9	C-10
Δδ, **1a → 1b**	+1.4	+6.4	+3.3	+8.9	−0.8	+3.4
Δδ, **2a → 2b**	+1.5	+4.8	+3.8	+10.6	−0.4	+2.7
Δδ, **3a → 3b**	+1.2	+6.6	+3.6	+9.4	−1.0	+4.1
Δδ, phenol → anisole[b]	−0.2	−1.3	+5.3	−1.3	−0.2	−0.3

[a] All chemical shift changes were determined from proton-decoupled spectra for compounds dissolved in deuteriochloroform; negative values are upfield changes. [b] Phenol and anisole were numbered to correspond to other compounds.

Chemical Shift Assignments for the Carbonyl Carbons 1, 4, and 11. Carbon 11 is easily assigned due to its large one-bond coupling to H-11 (Table VI). The quinoid carbonyls in **1a** and **1b** are distinguished by the three-bond couplings of carbon 1 to H-3 and H-15 (Figure 1b). Carbonyl carbon 4 is not appreciably coupled to either H-3 or H-15 and appears as a sharp singlet.

In **3a, 3b,** and **3c,** carbons 1 and 4 are no longer conjugated quinoid carbons and as a result are deshielded by approximately 20 ppm compared to the carbonyls of **1a** and **1b.** Further, since carbons 2 and 3 are now tetrahedral carbons, long-range coupling to H-3 and H-15 gives unresolved multiplets rather than well-defined coupling patterns (Figure 5b). The relative intensities of the resonances due to carbons 1 and 4 in the coupled spectrum allow qualitative chemical shift assignment. The downfield resonance at δ 202.3 is broader than the upfield resonance at δ 198.8 and may be tentatively assigned to carbon 1. It is expected that carbon 1 will be appreciably coupled through three bonds to the hydrogens on the angular methyl group and to H-3, resulting in a broader unresolved multiplet. This assignment is confirmed through the irradiation of the methyl hydrogens (Figure 5c). Although the resonance assigned to carbon 1 is not completely collapsed because it is still coupled to H-3, it is considerably narrower and grows in relative intensity compared to the resonance assigned to carbon 4.

The Stereochemistry of the Cyclohexadione Ring in 3a, 3b, and 3c. The angle of twist, θ, that the carbonyl group of a phenyl ketone makes with the aromatic ring (**9**) may be calculated from eq 1, where δ_c is the carbon-13 chemical shift



9

$$\cos^2\theta = 216.0 - \delta_c/20 \quad (1)$$

of the carbonyl carbon.[19,20] The angles calculated by this empirical method have been compared to those derived from ultraviolet and dipole moment studies of substituted phenyl ketones. The x-ray crystal structure of **3c** showed that the carbonyl groups lie above and below the plane of the aromatic ring. From these data it was found that carbons 1 and 4 make angles of 23.9 and 33.2° with respect to the aromatic ring.[5] Based on these angles, the calculated chemical shifts of car-

Table VI. Carbons 1, 4, and 11: Chemical Shifts and Proton–Carbon Couplings[a]

Compd	C-1 Shift, δ	$^1J_{H^1}$	$^3J_{H^3}$	$^3J_{H^{15}}$	C-4 Shift, δ	C-11 Shift, δ	$^1J_{H^{11}}$
1a	186.2		9.2	3.9	184.8	197.3	196.1
b	186.8		9.6	3.7	185.9	198.0	193.9
2a	114.4	156.1	u	u	152.3	198.4	189.9
b	115.5	157.1	u	u	152.2	198.5	191.3
c	115.8	157.3		5.5	150.2	198.4	189.5
3a	202.3		u	u	198.8	197.7	191.9
b	201.7		u	u	198.7	197.1	191.0
c	202.5		u	u	198.4	197.9	193.4

[a] Chemical shifts are in ppm downfield from Me_4Si using central resonance of $CDCl_3$ as an internal reference; solvent for **1b, 3a, 3b,** and **3c** was deuteriochloroform and solvent for **1a, 2a, 2b,** and **2c** was acetone; coupling constants are in Hz (±0.5 Hz); u, unresolved multiplet.

Table VII. Calculated Angles of Twist, θ, for Carbons 1 and 4

Compd	C-1, deg	C-4, deg
3a	34.1	22.0
b	31.9	21.6
c	34.8	20.3

bons 1 and 4 are δ 199.3 and 202.0, respectively. These are in good agreement with the observed values (δ 198.4 and 202.5) and confirm our earlier tentative chemical shift assignments. The calculated angles of twist for **3a, 3b,** and **3c** are shown in Table VII. It is seen that there is no significant change in these angles and that substituents on the cyclohexene ring do not appreciably change the conformation.

Experimental Section

Materials. All the compounds studied were isolated from 2–3-day-old cotton bolls and purified as described elsewhere: **1a,**[8] **1b,**[8] **2a,**[21] **2b,**[21] **2c,**[22] **3a,**[6] **3b,**[8] and **3c.**[7] Reagent grade deuteriochloroform (99.8 atom % D) and acetone were used for all NMR spectra. For proton-coupled carbon-13 spectra determined in acetone, approximately 15% by volume deuteriochloroform was added to provide a lock signal.

Carbon-13 Spectra. All carbon-13 spectra were obtained with a JEOL PFT-100 Nicolet 1080 Fourier transform spectrometer. Sample concentrations were in the range between 0.5 and 1.0 M. Proton-decoupled spectra required 1500–3000 average transients to obtain satisfactory signal to noise ratios. Proton-coupled and single frequency decoupled spectra required 6000–15000 average transients. Chemical shifts of the same compound at different concentrations in the same solvent were reproducible within ±1 Hz. Therefore, in the determination of the deuterium isotope shifts, chemical shift changes of less than ±1 Hz were considered negligible (Table III). The sweep width used for the coupled and single frequency decoupled spectra was selected to give the maximum resolution using 16K data points, to prevent "foldover" from either the alkyl or the carbonyl regions of the spectra, and ranged from 3500 to 4000 Hz. The precision of the coupling constant measurements was limited by the digital resolution, typically 4000 Hz/8000 data points = 0.05 Hz. For this reason, couplings of less than about 1.5 Hz were not resolved. Couplings in the range of 1.5 to 2.5 Hz occasionally appeared only as broadened resonances. For example, in the proton-coupled spectrum of **3a** the resonance at δ 148.3 is a doublet of doublets due to the four-bond coupling of H-11 to carbon 7. The downfield doublet is resolved ($^4J_{H^{11}}$ = 2.4 Hz), but the upfield peak is a broadened singlet (Figure 5b).

The carbon-13 chemical shift assignments for the alkyl regions were based upon decoupled and off-resonance coupled spectra and are reported in ppm downfield from Me_4Si using the central resonance of $CDCl_3$ as an internal reference (δ 76.9). For the chemical shift changes presented in Table V, the aryl, carbonyl, C-2, and C-3 shifts for **1a, 2a,** and **2b** were determined from proton-decoupled spectra for these compounds dissolved in deuteriochloroform rather than acetone and these shifts are included below. Small chemical shift changes may be noted compared to the chemical shifts determined in acetone (Tables I, II, IV, and VI).

1a:[5] δ 187.3 (C-1), 148.9 (C-2), 133.8 (C-3), 187.3 (C-4), 115.6 (C-5), 152.0 (C-6), 149.1 (C-7), 141.5 (C-8), 127.5 (C-9), 127.1 (C-10), 198.7 (C-11), 28.4 (C-12), 19.6 (C-13, C-14), 16.3 (C-15).

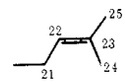
1b:[8] δ 28.7 (C-12), 20.8 (C-13, C-14), 16.3 (C-15).

2a: δ 116.7 (C-1), 133.9 (C-2), 113.1 (C-3), 151.7 (C-4), 111.6 (C-5), 155.6 (C-6), 142.7 (C-7), 134.3 (C-8), 129.4 (C-9), 114.3 (C-10), 199.4 (C-11), 27.8 (C-12), 20.1 (C-13, C-14), 21.4 (C-15).

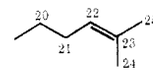
2b: δ 117.6 (C-1), 133.6 (C-2), 114.5 (C-3), 151.8 (C-4), 113.1 (C-5), 160.4 (C-6), 146.5 (C-7), 144.9 (C-8), 129.0 (C-9), 117.0 (C-10), 199.2 (C-11), 27.8 (C-12), 21.6 (C-13, C-14), 21.6 (C-15), 60.9 (C-16, OCH_3).

2c: δ 27.8 (C-12), 20.2 (C-13, C-14), 20.2 (C-15).

3a:[6] δ 28.8 (C-12), 19.8 and 19.6 (C-13, C-14), 23.6 (C-15), 32.3 (C-16), 118.6 (C-17), 135.0 (C-18), 39.5 (C-19), 21.4 (C-20, $R_3 = CH_3$), 27.6 (C-21, R_2 = chain A), 123.3 (C-22), 133.2 (C-23, R_2 = chain A), 25.6 (C-24, R_2 = chain A), 17.6 (C-25, R_2 = chain A).



Chain A



Chain B

3b:[8] δ 29.1 (C-12), 20.9 and 21.0 (C-13, C-14), 23.0 (C-15), 60.4 (C-26, OCH_3), 32.4 (C-16), 118.4 (C-17), 134.8 (C-18), 38.9 (C-19), 21.3 (C-20, $R_3 = CH_3$), 27.4 (C-21, R_2 = chain A), 123.3 (C-22, R_2 = chain A), 133.3 (C-23, R_2 = chain A), 25.6 (C-24, R_2 = chain A), 17.6 (C-25, R_2 = chain A).

3c:[5] δ 29.0 (C-12), 19.8 (C-13, C-14), 22.3 (C-15), 32.1 (C-16), 117.6 (C-17), 134.4 (C-18), 26.6 (C-19), 37.1 (C-20, R_3 = chain B), 26.0 (C-21, R_3 = chain B), 123.7 (C-22, R_3 = chain B), 131.4 (C-23, R_3 = chain B), 25.6 (C-24, R_3 = chain B), 17.6 (C-25, R_3 = chain B).

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Registry No.—Salicylaldehyde, 90-02-8; phenol, 108-95-2; anisole, 100-66-3.

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(15) The effect of strong intramolecular hydrogen bonding of the C-6 hydroxy proton to the aldehyde oxygen is also seen in the ^{1}H NMR shifts. In deuteriochloroform the C-6 hydroxy protons are far downfield (**1a**, δ 12.95; **1b**, δ 12.65; **2a**, δ 14.85; **2b**, δ 14.05; **2c**, δ 14.84; **3a**, δ 12.85; **3b**, δ 12.71; **3c**, δ 12.86). In contrast, the C-7 and C-4 hydroxy protons are observed between δ 5.80 and 6.60 (**1a**, δ 6.50; **2a**, δ 6.00 and 6.10; **2b**, δ 6.51; **2c**, δ 6.31 and 5.81; **3a**, δ 6.53; **3c**, δ 6.60).
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Optical Resolution Studies of Cyclophosphamide

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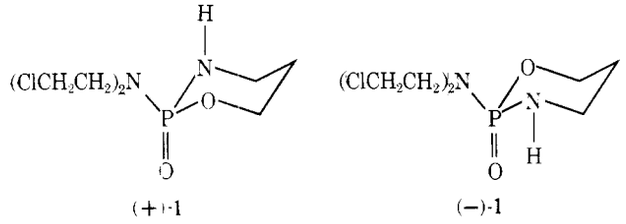
Cyclophosphamide (1) has been resolved by fluoride displacement (with c-$C_6H_{11}NH_3F$) of the optically active α-NpPhMeSi group of each chirality from the endocyclic nitrogen of the cyclophosphamide moiety in α-NpPhMeSi-c-$(NCH_2CH_2CH_2OP(O))N(CH_2CH_2Cl)_2$ ((−)-*S*(P)-*R*(Si)-**4**) and (+)-*R*(P)-*S*(Si)-**4**. The latter enantiomers were separated in high purity from their diastereomers ((+)-*R*(P)-*R*(Si)-**5** and (−)-*S*(P)-*S*(Si)-**5**, respectively) by recrystallization. The enantiomeric **5** derivatives could not be purified of **4** by a variety of techniques. Each diastereomeric mixture of **4** and **5** was synthesized by allowing the lithium salt of **1** (formed from **1** and *n*-BuLi) to react with (−)- and (+)-α-NpPhMeSiCl at low temperature. A simple method for the synthesis of anhydrous crystalline (±)-**1** is also described. The separation of the enantiomeric NH protons of anhydrous (±)-**1** in benzene-d_6 observed in the presence of the chiral shift reagent EuOpt offers a convenient method for estimating the optical purity of the enantiomers.

Introduction

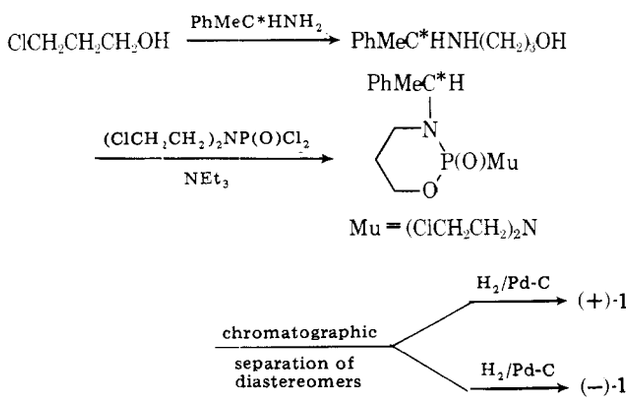
Racemic cyclophosphamide hydrate (Cytoxan, 2-[bis(2-chloroethyl)amino]-2-oxo-1,3,2-oxazaphosphorinane, (±)-**1**) is very effective in the clinical control of a variety of cancers.[1] After our efforts to resolve (±)-**1** were underway, Stec and



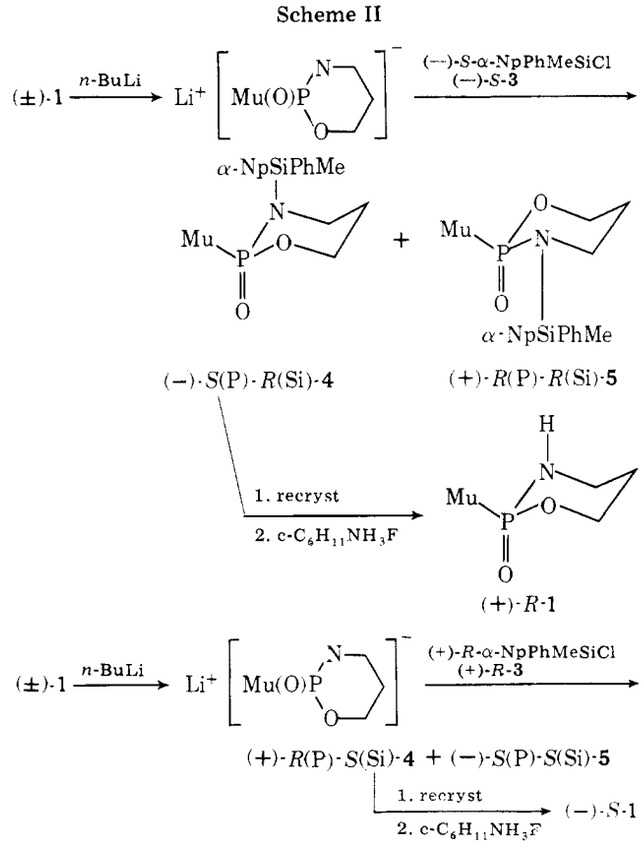
co-workers[2] published their approach to this problem which is outlined in Scheme I. In collaboration with Cox et al.[3] they showed that (−)-**1** is more efficacious against PC6 mouse tumors and the latter group demonstrated that (+)-**1** is preferentially metabolized in human patients.[3] Recently, the absolute configuration of (+)-**1** was shown to be *R* from the anomalous dispersion of x rays from the chlorine and phosphorus atoms.[4]

In view of the possibility that metabolic selectivity for a particular enantiomer may depend on the biological system under consideration, we decided to pursue the path outlined in Scheme II, since it would be of valuable aid in our current

Scheme I



Scheme II



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