Conversion of (*S*)-Allethrolone to Pyrethrin I, Jasmolin I, Cinerin I, and [*propenyl*-3-^{13}C]- and [*propenyl*-3-^{14}C]-(*S*)-Bioallethrin

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Pyrethrin I, jasmolin I, and cinerin I of high stereochemical purity were prepared from (*S*)-allethrolone by the following reaction sequence: protection of the hydroxyl and reduced carbonyl groups as their acetate and tetrahydropyranyl derivatives, respectively; ozonolysis to convert the propenyl compound to a key formylmethyl intermediate; Wittig reaction of this aldehyde with ylides derived from allyl-, *n*-propyl-, or ethyltriphenylphosphonium halide; cleavage of the tetrahydropyranyl ether and oxidation of the cyclopentenol to the cyclopentenone with pyridinium chlorochromate; separation of the *Z* and *E* isomers of the rethronyl acetates, hydrolysis, and esterification of the optically pure pyrethrolone, jasmolone, and cinerolone with (1*R*)-*trans*-chrysanthemoyl chloride. An extension of this synthesis via 3-[(1*R*)-*trans*-chrysanthemyloxy]-2-methyl-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]-1-cyclopentene-1-acetaldehyde as the critical intermediate allows isotopic label introduction by Wittig reaction near the terminal step, as illustrated with [*propenyl*-3-^{13}C]- and [*propenyl*-3-^{14}C]-(*S*)-bioallethrin of high isomeric and radiochemical purities. Stereochemical assignments are supported by appropriate optical rotation values and complete ^{1}H and ^{13}C NMR data.

Natural pyrethrins and optically pure synthetic analogues both unlabeled and with ^{13}C and ^{14}C labeling are needed for studies on their environmental degradation, metabolism, and mode of action. A variety of synthetic procedures have been used for this purpose. Radiosyntheses are reported for [^{14}C]- and [^{3}H]-(*S*)-bioallethrin (**1a**) and -pyrethrin I [(*Z*)-**1b**] prepared from [^{14}C]-(1*R*)-*trans*-chrysanthemic acid (Nishizawa and Casida, 1965; Yamamoto and Casida, 1968) and [^{3}*H*]-(*S*)-allethrolone and -pyrethrolone (Elliott and Casida, 1972) and for [^{14}C]-bioallethrin from [^{14}C]-(*RS*)-allethrolone (Yamamoto and Casida, 1968). Unlabeled (*RS*)-allethrolone has been converted to (*RS*)-jasmolone and -cinerolone (Pattenden and Storer, 1974) and to (*RS*)-pyrethrolone and (*RS*)-pyrethrin I (Sasaki et al., 1979) by protection of the hydroxyl or hydroxyl and carbonyl functions, oxidative cleavage of the propenyl group, and reconstruction of the butenyl, pentenyl, and pentadienyl substituents by a Wittig reaction. The present study develops methods for conversion of (*S*)-allethrolone (**2a**) (Martel et al., 1980) to (*Z*)-**1b**, jasmolin I [(*Z*)-**1c**], cinerin I [(*Z*)-**1d**], and [^{13}C]- and [^{14}C]-(*S*)-bioallethrin (Figure 1).

MATERIALS AND METHODS

Chemicals and Chromatography. (*S*)-Allethrolone ($[\alpha]^{22}_{D}$ = +14.7°, 1.0% in $CHCl_3$) and (1*R*)-*trans*-chrysanthemic acid ($[\alpha]^{22}_{D}$ = +25.6°, 1.0% in $CHCl_3$) were supplied as pure samples (>99.5%) by Roussel-Uclaf (Paris, France). [^{13}C]Methyl iodide (99% enrichment) for preparation of [^{13}C]methyltriphenylphosphonium iodide was from Prochem (Summit, NJ). [^{14}C]Methyltriphenylphosphonium iodide (1.0 mCi/mmol) was obtained from California Bionuclear Corp. (Sun Valley, CA).

Column chromatography used silica gel developed with hexane–acetone mixtures (referred to as silica gel column purification). Geometrical isomers of (*ZE*)-**3c** and (*ZE*)-**3d** were then separated on columns of silica gel impregnated with 20% $AgNO_3$ [silica gel ($AgNO_3$)] and developed with hexane–benzene mixtures. Preparative thin-layer chromatography (TLC) on silica gel F_{254} chromatoplates utilized hexane–acetone mixtures (5:1∼2:1) (system A) for all compounds followed, in the case of **1a** and (*Z*)-**1b–d** only, by repurification with toluene–ethyl acetate (6:1) (system B). TLC on $AgNO_3$-impregnated silica gel plates [TLC ($AgNO_3$)] (from dipping in 10% aqueous $AgNO_3$ and drying in the dark) involved development with toluene–ethyl acetate mixtures for analysis of *ZE* mixtures of **1c**, **1d**, **3c**, and **3d**.

Spectroscopy. IR spectra were recorded as thin films with the Perkin-Elmer 457 grating spectrometer. NMR spectra were obtained for samples in $CDCl_3$ with tetramethylsilane (Me_4Si) as the internal standard at 250 MHz (^{1}H) or 63 MHz (^{13}C) with the UCB-250 instrument (Chemistry Department, University of California, Berkeley). Optical rotations were measured at 22 °C with a cell path length of 100 mm (capacity ∼2 mL) by using a Perkin-Elmer 241 polarimeter.

^{1}H and ^{13}C NMR data are given in Table I for the rethronyl acetates and [*propenyl*-3-^{13}C]-(*S*)-bioallethrin and in Tables I–III of the supplementary material (see paragraph at end of paper regarding supplementary material) for all compounds. Optical rotation values are reported in Table II and IR data in Table I of the supplementary material.

RESULTS

Synthetic Routes. Two schemes were used. Figure 2 gives the preferred route for conversion of (*S*)-allethrolone to pyrethrins (*Z*)-**1b–d** via rethronyl acetates (*Z*)-**3b–d**. Figure 3 indicates a modification allowing isotopic label introduction near the terminal step by Wittig reaction of aldehyde **12**.

Protection of Carbonyl Group. Protection of the C(4) carbonyl is required to prevent any possible side reactions with Wittig reagents at this site and to deactivate asymmetric center C(1). (*RS*)-Allethrolone has been protected as its ketal by treatment with propylene oxide (Sasaki et al., 1979); however, the conditions required for removal of this group (ca. 3% H_2SO_4 in aqueous acetone for 1 week at room temperature) might be too harsh for retention of optical purity. This difficulty was overcome by reduction to a hydroxyl group and protection with a substituent, allowing easy reversion to the carbonyl group. Thus, (*S*)-allethrolone (**2a**) was acetylated and the acetate (**3a**) was reduced with $NaBH_4$ in an 2-propanol–methanol mixture to yield (1*S*,4*R*)- and (1*S*,4*S*)-**4a** as a mixture of

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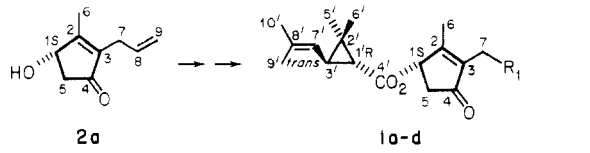
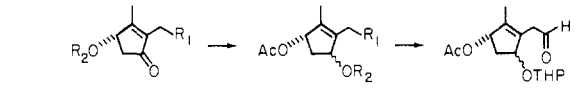


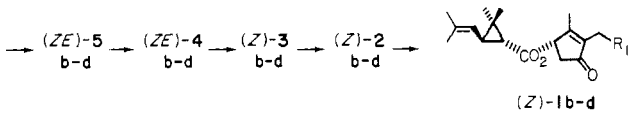
2a 1a-d

R_1: a. $-CH=CH_2$, $-CH=^{13}CH_2$, $-CH=^{14}CH_2$; b. $-CH=CHCH=CH_2$; c. $-CH=CHCH_2CH_3$; d. $-CH=CHCH_3$

Figure 1. Conversion of (*S*)-allethrolone (**2a**) to pyrethrin I [(*Z*)-**1b**], jasmolin I [(*Z*)-**1c**], cinerin I [(*Z*)-**1d**] and [*propenyl*-3-^{13}C]- and [*propenyl*-3-^{14}C]-(*S*)-bioallethrin (**1a**). The indicated numbers are used for the rethrins and equivalent positions in intermediates or related compounds.



2a R_2 = H; 3a R_2 = Ac 4a R_2 = H; 5a R_2 = THP 6



(*ZE*)-5 b-d → (*ZE*)-4 b-d → (*Z*)-3 b-d → (*Z*)-2 b-d → (*Z*)-1b-d

Figure 2. Conversion of (*S*)-allethrolone (**2a**) via rethronyl acetates [(*Z*)-**3b–d**] to the natural pyrethrins [(*Z*)-**1b–d**]. R_1 substituents are the same as those in Figure 1.

epimers (4:1 by ^{1}H and ^{13}C NMR, Tables I and II in the supplementary material) [e.g., the C(1) and C(4) protons exhibit distorted double doublet signals centered respectively at δ 5.39 and 4.50 for the (1*S*,4*R*)-alcohol and at δ 5.69 and 4.78 for the (1*S*,4*S*)-alcohol]. No reduction occurred at the conjugated double bond. Allylic alcohol **4a** was further converted to tetrahydropyranyl (THP) ether **5a** by using the mild catalyst pyridinium *p*-toluenesulfonate (Miyashita et al., 1977).

Conversion of Propenyl Substituent to Butenyl, Pentenyl, and Pentadienyl Groups. These reactions utilized **5a** and the general sequence of Sasaki et al. (1979). Key aldehyde **6** was prepared by ozonolysis of **5a** followed by workup with triphenylphosphine in CH_2Cl_2, in this case a more efficient procedure than the alternative oxidative cleavage with OsO_4–$NaIO_4$. Wittig reaction of aldehyde **6** with the ylide derived from allyltriphenylphosphonium bromide using *n*-butyllithium as the base in tetrahydrfuran (THF) produced (*ZE*)-**5b** which was deprotected by *p*-toluenesulfonic acid in ethanol to give alcohol (*ZE*)-**4b**. Oxidation with pyridinium chlorochromate (PCC) in CH_2Cl_2 yielded ketone (*ZE*)-**3b**. The same procedure was adapted for (*ZE*)-**3c** and (*ZE*)-**3d** derived from *n*-propyl- and ethyltriphenylphosphonium iodides, respectively.

Geometrical Isomer Composition and Resolution of Rethronyl Acetates. The *Z*/*E* ratios (^{1}H NMR) were 1/1 for **3b**, 7/1 for **3c**, and 4/1 for **3d** based primarily on the C(7)H_2 signals for (*ZE*)-**3b**, the C(11)H_3 signals for (*ZE*)-**3c**, and the C(7)H_2 and C(10)H_3 signals for (*ZE*)-**3d**. Table I shows the assignments of each proton of the 3 substituents of these esters. The *Z*/*E* ratios for **3b–d** were also evident by ^{13}C NMR, i.e., signals of C(2), C(3), and C-(7)–C(11) were distinguishable for the two geometrical isomers (Table I).

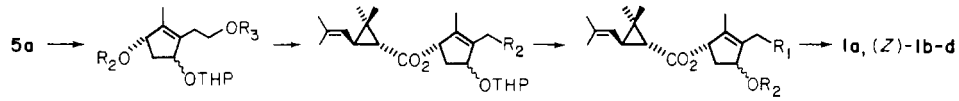
TLC ($AgNO_3$) with ethyl acetate–toluene (3:2) gave R_f values as follows: 0.37 for **3a**; 0.47 for both (*Z*)- and (*E*)-**3b**; 0.54 for (*Z*)-**3c** and 0.60 for (*E*)-**3c**; 0.49 for (*Z*)-**3d** and 0.57 for (*E*)-**3d**. Column chromatography of *ZE* mixtures on silica gel ($AgNO_3$) gave pure (*Z*)-**3c** and (*Z*)-**3d** but did not resolve (*ZE*)-**3b**. Fortunately, (*E*)-**3b** was easily removed as an adduct by selective reaction with tetracyanoethylene (Sasaki et al., 1979) to recover (*Z*)-**3b** by preparative TLC system A.

Conversion of Rethronyl Acetates to Rethrins and Examination of Stereochemical Purity. Rethronyl acetates (*Z*)-**3b–d** were hydryolyzed under mild conditions (K_2CO_3 in methanol) to rethrolones (*Z*)-**2b–d** which were esterified with (1*R*)-*trans*-chrysanthemoyl chloride to obtain the corresponding pyrethrins (*Z*)-**1b–d** with retention of stereochemistry (Table II) and with appropriate ^{1}H NMR spectral data [Table I of the supplementary material; for a comparison see Bramwell et al. (1969)] and ^{13}C NMR parameters [Tables II and III of the supplementary material; for a comparison see Crombie et al. (1975)]. The overall reactions therefore proceeded without epimerization at C(1).

(*Z*)-**1b** and its 1*R* diastereoisomer [(1*R*,*Z*)-**1b**] are distinguishable in a mixture by ^{1}H NMR as follows. For (*Z*)-**1b**, δ 1.14, 1.26, and 2.04 [C(6′)H_3, C(5′)H_3, and C-(6)H_3], δ 2.23 and 2.87 [C(5)H_2], and δ 5.66 [C(1)H_β]. For (1*R*,*Z*)-**1b**; δ 1.15, 1.29, and 2.02 [C(6′)H_3, C(5′)H_3, and C(6)H_3], δ 2.29 and 2.85 [C(5)H_2], and δ 5.75 [C(1)H_α]. ^{13}C NMR experiments also confirmed the C(1) configuration, as ^{13}C NMR of (1*RS*,*Z*)-**1b** gave two lines for each carbon of the methylcyclopentenolone skeleton as follows: C(1) 72.61 (*R*), 72.95 (*S*); C(2) 165.50 (*R*), 165.38 (*S*); C(3) 141.90 (*R*), 141.96 (*S*); C(4) 203.71 (*R*), 203.77 (*S*); C(5) 41.60 (*R*), 42.05 (*S*); C(6) 14.01 (*R*), 14.12 (*S*).

Preparation of Aldehyde 12. The synthetic route in Figure 2 with appropriate ^{14}C-labeled Wittig reagents provides access to [^{14}C]rethrins but should ideally be modified to allow ^{14}C incorporation at a later step in the pathway. This was accomplished by using aldehyde **12** instead of **6** for the Wittig reaction (Figure 3).

Aldehyde **12** was not obtained directly from **1a** since, as might be expected (Crombie et al., 1970; Ruzo et al., 1982), neither ozone nor OsO_4 selectively attacked the terminal double bond in the alcohol moiety; the same difficulty was encountered with allethrin analogue **13a** prepared by $NaBH_4$ reduction of **1a** to **14a** and THP protection of the resulting hydroxyl group. Aldehyde **6** was so unstable that **12** was not obtained by attempted mild base hydrolysis of the acetyl group and esterification with chrysanthemoyl chloride. A more indirect route via



5a → 1a, (*Z*)-1b-d

7 R_2 = Ac; R_3 = H 10 R_2 = CH_2OAc 13a, (*ZE*)-13b-d R_2 = THP

8 R_2 = H; R_3 = H 11 R_2 = CH_2OH 14a, (*ZE*)-14b-d R_2 = H

9 R_2 = H; R_3 = Ac 12 R_2 = CHO

Figure 3. Route for isotopic synthesis of rethrins allowing label introduction near the terminal step by Wittig reaction of aldehyde **12**. R_1 substituents are the same as those in Figure 1.

Table I. ^1H and ^{13}C NMR Data for 3 Substituents of Pyrethronyl Acetate [(*Z*)-3b], Jasmolonyl Acetate [(*Z*)-3c], Cineronyl Acetate [(*Z*)-3d], Their *E* Isomers, and [*propenyl*-3-^{13}C]-(*S*)-Bioallethrin

	7 8 9 10 11 $-CH_2CH=CHCH=CH_2$		7 8 9 10 11 $-CH_2CH=CHCH_2CH_3$		7 8 9 10 $-CH_2CH=CHCH_3$		7 8 9 $-CH_2CH=C^*H_2$
position	(*Z*)-3b	(*E*)-3b	(*Z*)-3c	(*E*)-3c	(*Z*)-3d	(*E*)-3d	1a (* = ^{13}C, 99% enrichment)
			^1H NMR, Chemical Shifts (δ) and Coupling Constants (*J*, Hz)[a]				
C(7)H_2	3.12 (d), *J* = 7.5	3.01 (d), *J* = 6.5	2.98 (d), *J* = 7	2.92 (d), *J* = 6	2.99 (d), *J* = 7	2.91 (d), *J* = 6	2.99 (d), *J* = 6.5
C(8)H	5.34 (dt), *J* = 11, 7.5	5.63 (dt), *J* = 15.5, 6.5	5.23 (dtt), *J* = 10.5, 7, 1.5	5.33 (dtt), *J* = 15.5, 6, 1	5.29 (dtq), *J* = 10.5, 7, 1.5	~5.4 (m)	5.77 (ddt), *J* = 17, 11, 6.5
C(9)H_n	6.03 (dd), *J* = 11, 11	6.05 (dd), *J* = 15.5, 11	5.42 (dtt), *J* = 10.5, 7, 1.5	5.49 (dtt), *J* = 15.5, 6, 1	5.51 (dqt), *J* = 10.5, 6.5, 1.5	~5.4 (m)	5.01 (cis) (ddd), $J_{C,H}$ = 158, $J_{H,H}$ = 11, 1.5 5.02 (trans) (ddd), $J_{C,H}$ = 154, $J_{H,H}$ = 17, 1.5
C(10)H_n	6.76 (ddd), *J* = 17, 11, 11	6.27 (ddd), *J* = 17, 11, 11	2.16 (qd), *J* = 7.5, 7	1.99 (qd), *J* = 7.5, 6	1.71 (d), *J* = 6.5	1.63 (d), *J* = 5	
C(11)H_n	5.18 (cis) (dd), *J* = 11, <1 5.23 (trans) (dd), *J* = 17, <1	4.99 (cis) (dd), *J* = 11, <1 5.11 (trans) (dd), *J* = 17, <1	0.99 (t), *J* = 7.5	0.95 (t), *J* = 7.5			
			^{13}C NMR, Chemical Shifts (ppm from Me_4Si)[a]				
C(2)	165.0	165.3	164.3	164.7	164.4	164.8	165.9
C(3)	142.0	141.6	142.9	142.9	142.8	142.3	141.4
C(7)	21.7	26.0	21.2	26.1	21.0	26.0	27.2
C(8)	126.8	129.4	123.9	123.7	125.4	126.1	133.6 (d, $J_{C,C}$ = 71)
C(9)	130.4	132.3	133.2	133.8	125.6	126.5	115.9
C(10)	131.6	136.6	20.6	25.4	12.8	17.8	
C(11)	118.3	116.1	14.1	13.6			

[a] Spectra measured in $CDCl_3$ with Me_4Si as the internal standard.

Table II. Optical Rotation Data for Rethrolones, Rethronyl Acetates, and Rethrins Prepared from Aldehyde 6

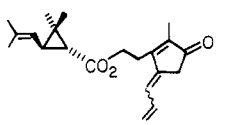
	$[\alpha]^{22}_D$, deg (% in $CHCl_3$)		
(S)-alcohol series	rethrolone	rethronyl acetate	rethronyl (1R)-*trans*-chrysanthemate
allethrolone	+14.1 (1.2)[a]	+27.7 (1.0)[b]	−23.4 (1.0)[c,d]
pyrethrolone	+15.6 (2.1)	+30.3 (1.6)	−17.5 (1.4)[d]
jasmolone	+15.6 (1.3)	+29.2 (1.7)	−17.1 (1.2)
cinerolone	+16.3 (1.3)	+31.4 (1.7)	−17.4 (1.2)

[a] (S)-Allethrolone (99.5%) from Roussel-Uclaf gave $[\alpha]^{22}_D$ +14.7° (1.0). [b] From acetylation of Roussel-Uclaf (S)-allethrolone. [c] (S)-Bioallethrin (>99%) from column chromatography of a Roussel-Uclaf sample. [d] $[\alpha]^{22}_D$ values for rethrins prepared from aldehyde 12 were −21.9° (1.0) for (S)-bioallethrin, −22.6° (2.6) for [*propenyl*-3-^{13}C]-(S)-bioallethrin, and −17.5° (1.0) for pyrethrin I.

alcohol 7 was therefore used to obtain 12 (Figure 3).

Primary alcohol 7 was prepared by reduction of 6 with $NaBH_4$ or direct ozonolysis of olefin **5a** followed by reduction of the ozonide with $NaBH_4$ in methanol. Replacement of the acetoxy group of 7 with the chrysanthemyloxy substituent required a protecting group (R_3) which is retained on hydrolysis of the acetate but can be selectively removed in the presence of the C(4) THP ether. The (β-methoxyethoxy)methyl (MEM) ether was evaluated as a potential candidate (Corey et al., 1976), but unfortunately the MEM ether of chrysanthemate 11 (prepared from the MEM ether of 7) was not deprotected to obtain alcohol 11 by using the recommended Lewis acid, either $ZnBr_2$ or $TiCl_4$. Finally, the key intermediate 11, which should be readily oxidized to aldehyde 12, was synthesized from 7 by a four-step procedure described below.

The acetoxy group of 7 was hydrolyzed with K_2CO_3 in methanol to give diol 8 which was selectively acetylated at the primary alcohol function to give secondary alcohol 9 on treatment with 1 equiv of acetic anhydride in pyridine. The isomeric monoacetates, secondary alcohol 9 and primary alcohol 7, are differentiated by their C(1)H NMR signals at δ 4.35 and 5.42 and their acetoxy methyl signals at δ 2.03 and 2.05, respectively. On the other hand the ^{13}C NMR spectra of THP ethers 7 and 9 were complicated because of the presence of four diastereoisomeric pairs. The signals of C(2), C(3), and C(5) have, however, clearly different shifts. Detailed NMR examination of crude 9 showed that there was contamination with ca. 5% 7. Compounds 7 and 9 were not separable by TLC, and further derivatives from 9 were contaminated with trace amounts of compounds originating from 7. The final product derived from impurity 7 [probably 15 in the



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synthesis of (ZE)-**1b**] was readily separated from the desired compound by TLC system B. The free hydroxyl group of 9 was esterified with (1R)-*trans*-chrysanthemoyl chloride in the presence of pyridine to give ester 10 which was selectively hydrolyzed by K_2CO_3 in methanol to give the key alcohol 11. PCC in CH_2Cl_2 oxidized alcohol 11 to aldehyde 12.

Conversion of Aldehyde 12 to Rethrins and [^{13}C]- and [^{14}C]-(S)-Bioallethrin. Wittig condensation with the ylide derived from the appropriate allyl- or alkyltriphenylphosphonium halide in THF gave olefins **13a** and (ZE)-**13b–d**. The THP protecting group was cleaved with *p*-toluenesulfonic acid to generate alcohols **14a** and (ZE)-**14b–d** which were oxidized with PCC to ketones **1a** and (ZE)-**1b–d**. NMR analyses revealed Z/E ratios of 1/1 for **1b**, 7/1 for **1c**, and 4:1 for **1d**; interestingly, these ratios are the same ones encountered with (ZE)-**3b–d** described above and for which there is precedent in analogous reactions (Pattenden and Storer, 1974; Sasaki et al., 1979; Székely et al., 1980).

TLC ($AgNO_3$) with toluene–ethyl acetate (1:1) gave R_f values as follows: 0.53 for **1a**; 0.58 for both (Z)- and (E)-**1b**; 0.64 for (Z)-**1c** and 0.67 for (E)-**1c**; 0.60 for (Z)-**1d** and 0.65 for (E)-**1d**. Thus, silica gel impregnated with $AgNO_3$ is appropriate to resolve pure (Z)-**1c** and (Z)-**1d** from their E isomers. Selective reaction between (E)-**1b** and tetracyanoethylene (Sasaki et al., 1979) and removal of this adduct by TLC system A afforded pure (Z)-**1b**.

The successful conversions above confirm that the stereochemistry of the chrysanthemate is not affected by the basic conditions of the Wittig reaction. The NMR data (Table I) and optical rotations (Table II) establish that every step proceeds without loss of chirality. The overall yields from the allyl- or alkyltriphenyl-phosphonium halides to rethrins (10–15%) were appropriate for isotropic labeling as established by preparation of both [*propenyl*-3-^{13}C]- and [*propenyl*-3-^{14}C]-(S)-bioallethrin. NMR analyses of [^{13}C]-**1a** gave reasonable coupling constants ($J_{C,H}$, $J_{C,C}$) as shown in Table I. The described procedure is also amenable to isotopic labeling of the natural esters from appropriate [^{13}C]- or [^{14}C]allyl- or -alkyltriphenylphosphinium halides.

EXPERIMENTAL SECTION

Conversion of (S)-Allethrolone via Rethronyl Acetates to Rethrins (Figure 2). (S)-Allethronyl acetate (**3a**) was prepared by dropwise addition of acetic anhydride (8 g, 78 mmol) to (S)-allethrolone (**2a**) (10 g, 66 mmol) in 20 mL of pyridine with stirring at 15–25 °C. This solution was stirred overnight at room temperature, then poured into water, and extracted with ether. The ether layer was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo (referred to below as the usual workup procedure). Distillation of the residue gave 12 g (62 mmol, 94%) of **3a**, bp 125 °C (3 mmHg).

Allylic alcohol **4a** was obtained on treatment of **3a** (12 g, 62 mmol) in 2-propanol (100 mL) with excess $NaBH_4$ (1.5 g, 39 mmol) while stirring below 30 °C. After 1 h methanol (10 mL) was added dropwise, stirring was continued for 15 h at 25 °C, and then the mixture was concentrated to ca. 30 mL in vacuo, and poured into 60 mL of water. Crude **4a** was extracted into ethyl acetate and washed with 1 N HCl, and after the usual workup and silica gel column purification it gave 9.6 g (49 mmol, 79%) of **4a** without detectable separation of the diasteroisomers.

THP ether **5a** was prepared by adding pyridinium *p*-toluenesulfonate (0.5 g) to a stirred solution of alcohol **4a** (9.6 g, 49 mmol) and dihydropyran (6.3 g, 75 mmol) in 100 mL of CH_2Cl_2. After 5 h of stirring at 25 °C, the usual workup and silica gel column purification gave 12 g (43 mmol, 87%) of **5a**.

Aldehyde **6** was obtained on introducing ozone into a solution of **5a** (670 mg, 2.4 mmol) in 50 mL of CH_2Cl_2 in a dry ice–acetone bath until **5a** disappeared (TLC). The resultant mixture was treated with triphenylphosphine (682 mg, 2.6 mmol) with stirring. The reaction temperature was gradually raised to 20 °C and stirring was continued for 3 h. After evaporation of CH_2Cl_2 **6** was extracted

with hexane–ether (2:1). Concentration of the solvent in vacuo gave **6** as a crude oil (ca. 700 mg), which was employed for the next step without further purification (NMR revealed a small amount of triphenylphosphine oxide contaminant).

THP intermediate **5b** was prepared by Wittig condensation of aldehyde **6** and the ylide obtained on injecting a solution of *n*-butyllithium in hexane (1.2 mL, 2.0 mmol) into a stirred suspension of allyltriphenylphosphonium bromide (770 mg, 2.0 mmol) in 50 mL of dry THF at 10 °C under dry nitrogen. After the mixture was stirred for 30 min at room temperature, the crude aldehyde **6** (ca. 700 mg) in 5 mL of dry THF was injected into the red solution of the ylide under 10 °C. The reaction mixture was stirred for 2 h at room temperature and poured into 100 mL of water. Crude (*ZE*)-**5b** was extracted with ethyl acetate, washed with 1 N HCl, and after the usual workup and chromatography on the silica gel column it gave 250 mg of pure (*ZE*)-**5b** (0.82 mmol, 34% from **5a**).

Pentadienyl alcohol (*ZE*)-**4b** was made by adding *p*-toluenesulfonic acid (10 mg) to the THP ether (*ZE*)-**5b** (250 mg, 0.82 mmol) in 5 mL of ethanol and stirring for 3 h at room temperature. The reaction mixture was poured into 20 mL of saturated aqueous $NaHCO_3$, and crude (*ZE*)-**4b** was recovered on extraction with ethyl acetate, drying, and concentration as above. Silica gel column purification gave 136 mg (0.61 mmol, 75%) of pure (*ZE*)-**4b**.

Ketone (*ZE*)-**3b** was obtained from alcohol (*ZE*)-**4b** (136 mg, 0.61 mmol) in 30 mL of CH_2Cl_2 on mixing with 100 mg of PCC and stirring at room temperature for 3 h. After filtration to remove the precipitate, the solvent was removed in vacuo and crude product (*ZE*)-**3b** was extracted with ether. Purification by TLC system A gave 110 mg (0.50 mmol, 82%) of pure 1/1 (*ZE*)-**3b**. This isomer mixture was treated with tetracyanoethylene (50 mg, 0.38 mmol) in 5 mL of THF at room temperature for 1 day. THF was removed in vacuo and the residue was chromatographed with TLC system A to give 48 mg (0.22 mmol, purification yield ca. 85%) of pure (*Z*)-**3b**. Analogous procedures on a 2-mmol scale gave 7:1 (*Z*)- and (*E*)-**3c** and 4:1 (*Z*)- and (*E*)-**3d** in ca. 20% overall yields from **5a**. Column chromatography on silica gel ($AgNO_3$) gave ca. 60 mg each of jasmolonyl acetate [(*Z*)-**3c**] and cineronyl acetate [(*Z*)-**3d**].

Pyrethrin I was prepared by hydrolysis of (*Z*)-**3b** (48 mg, 0.22 mmol) with K_2CO_3 (10 mg) in methanol (3 mL) for 2 h at room temp. to give 34 mg (0.19 mmol, 86%) of (*Z*)-**2b** recovered by addition of water, extraction with ethyl acetate, washing with 1 N HCl, workup in the usual manner, and TLC in system A. The recovered (*Z*)-**2b** was esterified with (1*R*)-*trans*-chrysanthemoyl chloride (35 mg, 0.19 mmol) in benzene (2 mL) containing pyridine (30 μL). After being stirred at room temperature for 6 h, the reaction mixture was washed with 1 N HCl. The usual workup and purification with TLC system A gave 45 mg (0.14 mmol, 73%) of pyrethrin I [(*Z*)-**1b**]. Similarly, hydrolysis yields for (*Z*)-**3c** and (*Z*)-**3d** were ca. 85% and esterification yields for (*Z*)-**2c** and (*Z*)-**2d** were ca. 75%.

Isotopic Labeling of Rethrins (Figure 3). Primary alcohol **7** was prepared by introducing ozone into a solution of olefin **5a** (8.4 g, 30 mmol) in 150 mL of methanol in a dry ice–acetone bath until **7** disappeared (TLC), then warming to 0 °C, and treating with excess $NaBH_4$ (1.7 g, 45 mmol) with stirring below 20 °C. After being stirred at room temperature for 2 h the mixture was concentrated to ca. 50 mL in vacuo and poured into 100 mL of water. Crude **7** was extracted with ethyl acetate, washed with 1 N HCl, and after the usual workup and silica gel column purification it gave 4.9 g (17 mmol, 58%) of pure **7**.

Diol **8** was obtained on stirring primary alcohol **7** (4.9 g, 17 mmol) in 75 mL of methanol containing K_2CO_3 (1.0 g) for 2 h at room temperature. Solvent evaporation in vacuo and silica gel column purification gave 3.6 g (15 mmol, 86%) of **8**.

Secondary alcohol **9** was prepared by dropwise addition of 1.6 g (16 mmol) of acetic anhydride to diol **8** (3.6 g, 15 mmol) in 30 mL of pyridine under stirring at room temperature. After being stirred overnight the solution was poured into water and extracted with ethyl acetate. The extract was washed with 1 N HCl and subjected to the usual workup and silica gel column purification to yield 2.9 g (10 mmol, 67%) of **9** (contaminated with ca. 5% **7**), 0.8 g of diacetate, and 0.3 g of the parent alcohol (**8**).

Acetate intermediate **10** was made by treating secondary alcohol **9** (2.9 g, 10 mmol) and 0.8 g of pyridine in 30 mL of benzene with 1.9 g (10 mmol) of (1*R*)-*trans*-chrysanthemoyl chloride. The reaction mixture was stirred at room temperature for 6 h, washed with 1 N HCl, and subjected to the usual workup and silica gel column purification to yield 3.3 g (7.6 mmol, 74%) of **10**.

Alcohol intermediate **11** was obtained (2.7 g, 6.9 mmol, 91%) on hydrolysis of **10** (3.3 g, 7.6 mmol) and chromatography in the same manner as that described for the preparation of **8**.

Aldehyde intermediate **12** was prepared by mixing alcohol **11** (470 mg, 1.2 mmol) in 50 mL of CH_2Cl_2 with 400 mg of PCC followed by stirring at room temperature for 3 h. After filtration to remove the precipitate, CH_2Cl_2 was evaporated in vacuo and the crude aldehyde **12** was extracted with hexane from the residue. Concentration of the solvent gave ca. 400 mg of **12** as a colorless oil, which was employed for the next step without further purification.

Pentadienyl intermediate (*ZE*)-**13b** was obtained by Wittig condensation between aldehyde **12** (ca. 400 mg) and the ylide derived from 380 mg (1.0 mmol) of allyltriphenylphosphonium bromide in an identical manner with that described for (*ZE*)-**5b**, giving 190 mg (0.46 mmol, 38% from **11**) of (*ZE*)-**13b**. Analogous procedures with *n*-propyl- and ethyltriphenylphosphonium iodides gave (*ZE*)-**13c** and (*ZE*)-**13d** in ca. 35% yields.

The alcohol precursors (*ZE*)-**14b–d** were obtained and oxidized to (*ZE*)-**1b–d** in the same manner described for (*ZE*)-**3b**. Preparative TLC system A furnished 89 mg [0.27 mmol, 58% from (*ZE*)-**13b**] of 1:1 (*ZE*)-**1b**. The *E* isomer was removed by the same method used for (*ZE*)-**3b** followed by repurification with TLC system B to give 39 mg (0.12 mmol, purification yield ca. 85%) of pure (*Z*)-**1b**. The yields of (*Z*)-**1c** and (*Z*)-**1d** mixed with small amounts of their *E* isomers were ca. 60% from (*ZE*)-**13c** and (*ZE*)-**13d**.

[^{13}C]Methyltriphenylphosphonium iodide and [^{14}C]-methyltriphenylphosphonium iodide were converted to [*propenyl*-3-^{13}C]- and [*propenyl*-3-^{14}C]-(*S*)-bioallethrin in overall yields from the phosphonium salt of ca. 15% and with a radiochemical purity for the ^{14}C-labeled compound of >99%.

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Registry No. **1a**, 28434-00-6; (1*R*)-**1a**, 61009-26-5; [^{13}C]-**1a**, 83478-01-7; [^{14}C]-**1a**, 83478-02-8; (*Z*)-**1b**, 121-21-1; (1*R*,*Z*)-**1b**, 83602-16-8; (*Z*)-**1c**, 4466-14-2; (*E*)-**1c**, 83541-03-1; (*Z*)-**1d**, 25402-

06-6; (*E*)-**1d**, 83602-15-7; **2a**, 22373-75-7; (*Z*)-**2b**, 487-67-2; (*Z*)-**2c**, 83541-04-2; (*Z*)-**2d**, 3894-82-4; **3a**, 22373-76-8; (*Z*)-**3b**, 22373-74-6; (*E*)-**3b**, 83572-03-6; (*Z*)-**3c**, 83541-05-3; (*E*)-**3c**, 83541-06-4; (*Z*)-**3d**, 83541-07-5; (*E*)-**3d**, 83541-08-6; (4*R*)-**4a**, 83478-03-9; (4*S*)-**4a**, 83541-09-7; **4b**, 83478-04-0; **5a**, 83486-42-4; **5b**, 83478-05-1; **6**, 83478-06-2; **7**, 83478-07-3; **8**, 83478-08-4; **9**, 83478-09-5; **10**, 83478-10-8; **11**, 83478-11-9; **12**, 83478-12-0; **13a**, 83478-13-1; **13b**, 83478-14-2; **13c**, 83478-15-3; **13d**, 83478-16-4; **14a**, 83478-17-5; **14b**, 83478-18-6; **14c**, 83478-19-7; **14d**, 83478-20-0; **15**, 83478-21-1; allyltriphenylphosphonium bromide, 1560-54-9; propyltriphenylphosphonium iodide, 14350-50-6; ethyltriphenylphosphonium iodide, 4736-60-1; (1*R*)-*trans*-chrysanthemoyl chloride, 4489-14-9; [^{13}C]methyltriphenylphosphonium iodide, 81826-67-7; [^{14}C]methyltriphenylphosphonium iodide, 1560-52-7.

Supplementary Material Available: IR and ^{1}H NMR data and ^{13}C NMR data supplemental to those in the text and in Table I (8 pages). Ordering information is given on any current masthead page.

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An Analysis of the Limonin and Naringin Content of Grapefruit Juice Samples Collected from Florida State Test Houses

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An analysis of the limonin and naringin content of 6685 grapefruit juice samples (representing approximately 6% of all grapefruit harvested) collected from three Florida State Test Houses showed that there were statistically significant differences between the Test Houses. There also was a statistically significant difference in the concentration of these compounds in the juice from the different cultivars of fruit sampled. Average limonin and naringin concentrations in the juice remained fairly constant through the beginning of the season until a freeze occurred in early 1981. Juice obtained from fruit harvested after this freeze contained decreasing amounts of limonin and increasing amounts of naringin through the remainder of the season. Results showed that there was no strong correlative relationship between limonin, naringin, Brix, percent acid, and Brix/acid ratio.

The bitterness in grapefruit and processed grapefruit products is primarily due to the presence of two compounds, limonin and naringin. Limonin is an intensely bitter triterpenoid dilactone derivative and is responsible for the "delayed bitterness" in processed citrus products. Naringin is the major flavonoid bitter compound occurring mainly in grapefruit and imparts an immediate bitterness to juice (Maier et al., 1977). Other important qualitative characteristics which affect the organoleptic properties include Brix, acid, and solid content in juice and processed products.

For a number of years accurate and simple tests have been employed for these other qualitative parameters. However, it has been only recently that the routine measurement of limonin and naringin became possible through the development of an accurate, simple, and specific radioimmunoassay (RIA) for limonin (Weiler and Mansell, 1980; Mansell and Weiler, 1980) and a RIA for flavonoid neohesperidosides (primarily naringin) (Jourdan et al., 1982a,b). These two tests have widened the scope of studies in citrus quality research and are presently being adapted for use as routine quality control assays in nonresearch applications. In this paper we present the results of analyses of the limonin and naringin content of juice from individual truckloads of grapefruit from three State Test Houses which were harvested from Oct 1980 through May 1981. The purpose of the present study was to determine whether the content of these two bitter principles was correlated with any of the other qualitative parameters which were being routinely assayed. In addition, we wanted to establish whether there was any relationship between bitter principle concentration and seasonality changes.

MATERIALS AND METHODS

Samples of grapefruit juice were obtained from State Test Houses located at three different processing plants in west-central Florida from Oct 1980 through May 1981. The juice was collected from the same batches used for the determination of Brix and acid and were collected by State Test House personnel. Samples were stored in 1.5-mL plastic vials which contained sodium azide to retard microbial growth, and each vial was labeled with the load number (representing a random sample from approxi-

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