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STRUCTURE OF STROBIC ACID

D. F. ZINKEL* and B. P. SPALDING[a]
Forest Products Laboratory,† Forest Service, USDA, Madison, Wis. 53705

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Abstract—Strobic acid, a new diterpene resin acid from *Pinus strobus*, has been shown to have structure **1a** by degradation and CD correlation.

In a preliminary communication,[1] we reported the isolation and partial characterization of a new diterpene resin acid found in the cortex and needles of eastern white pine, *Pinus strobus* L. We present here the details for the elucidation of the complete structure of this unusual resin acid, strobic acid (14*S*,17-cyclolabda-8(17),12-dien-18-oic acid, **1a**).‡

Consideration of the spectral data for methyl strobate suggested **1b** (with stereochemistry at C-14 not determined) as the likely structure. The basic skeleton was confirmed by exhaustive ozonolysis, Jones oxidation, and methylation to yield a product which was identical with the keto diester **2** prepared from methyl levopimarate. Thus, the double bond positions, the substituents on the A and B rings and the absolute stereochemistry at C-4, C-5, C-9, and C-10 are unambiguously confirmed. Only one arrangement of the remaining five carbon atoms in the other (unstable) ozonolysis fragment can be accommodated by the NMR data for the parent methyl strobate.

Examination of spectra did not reveal any information concerning the stereochemistry at C-14. The CD of **1b** and its corresponding alcohol, strobol,[3] have an interesting minima at 230·5 nm, $[\theta]$ of about $-5000°$, which could correspond to overlap of the non-conjugated π system; lacking appropriate model compounds, however, this is of little value in assigning the C-14 stereochemistry. It was hoped that the CD of the osmate ester of diol **3** (osmylation of **1b** should occur from the α side on steric considerations) could be used to establish the stereochemistry in the manner proposed by Scott and Wrixon.[4] Although the CD showed a minimum in the 500 nm region corresponding to a k′ conformation, an examination of models shows that both k and k′ conformations are possible for either C-14 epimer.

The C-14 stereochemistry was determined by conversion of **3** to keto diester **7b**, and by comparing the CD of **7b** with ORD data for methyl-α-methylalkyl ketones. Although diol **3** was readily cleaved to form **4**, hydrogenation of **4** did not proceed cleanly. The reverse order of preparation, however, was effective. Hydrogenation of diol **3** (or the 8-monoenoate from isomerization) in the presence of strong acid produced the saturated derivative **5**, which should have the thermodynamically more stable *trans-transoid-trans* skeleton. Cleavage of **5** with periodate yielded **6** which, on oxidation and subsequent methylation, gave **7b**. Comparison of the CD for **7b** ($[\theta]_{283}+3660°$] with ORD data[5] for *S*-3-methylpentan-2-one (γ substituents such as in 5-methylheptan-2-one make only minor contributions to the ORD curve) showed that the configuration of the secondary Me of **7b**, and thus the corresponding C-14 of the parent methyl strobate, is *S*.

Hydrogenation of methyl strobate produces four major components: dihydrostrobates **8** and **9** and tetrahydrostrobates **10** and **11**. In formulating tentative stereochemical assignments for the hydrogenation products, we presume that the formation of the 8(17)-enoate (**9**) occurs without change of configuration at C-14, and hydrogenation of the C-12 (C-13) double bond takes place from the α side. The stereochemistry at C-8 of the tetrahydrostrobates **10** and **11** and of dihydrostrobate **8** is assigned in analogy with observations on the tetrahydroabietates[6] and the tetrahydropimarates/tetrahydroisopimarates;[7] BC *cis* ring junction results in downfield shift of the C-10 Me hydrogens in the NMR spectra. It seems reasonable to assume a sequence of **1** → **9** → **10**, **11** as the primary hydrogenation pathway. This leads to retention of the *S* configuration at C-14 as tentatively assigned for **9**, **10**, and **11**.

EXPERIMENTAL

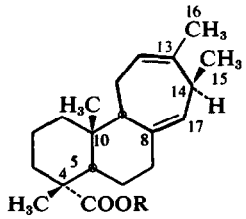
M.ps were determined in evacuated glass capillaries and are corrected. UV spectra were obtained for iso-

*To whom communications should be addressed.

[a]Present address, Dept. of Agronomy, Cornell Univ., Ithaca, New York 14850.

†Maintained in cooperation with the University of Wisconsin.

‡The systematic name for this acid was previously given as an *abeo*pimaradienoic acid.[1] However, the simpler and thus correct systematic name is based on a cyclized labdane skeleton.[2]



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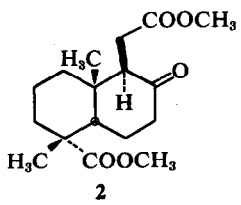
b: R = CH_3



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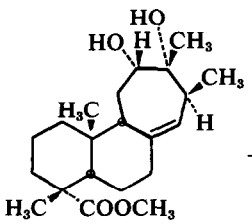

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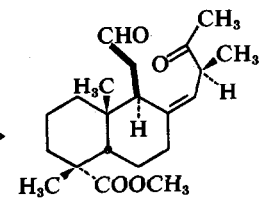

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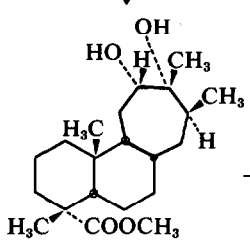

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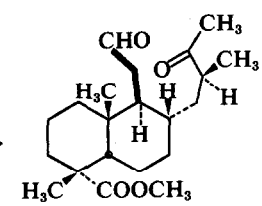

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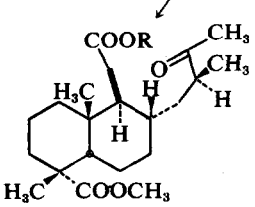
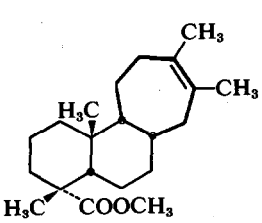


7a: R = H
b: CH_3



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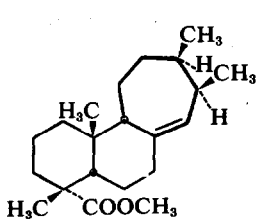

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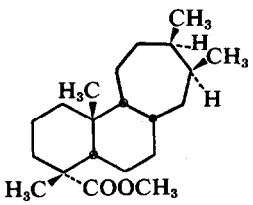

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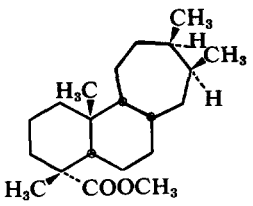

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octane solns (for conditions, see Table 2 of Ref 6). NMR spectra of $CDCl_3$ solns were recorded with a Varian HA-100 spectrometer using TMS as internal standard. (Complete NMR, IR, UV, and mass spectra for **1**, **8**, **9**, **10**, and **11** appear in Ref 8.) CD measurements were made with a Cary 60 spectropolarimeter.

Isolation of methyl strobate (**1**). Small scale isolation of methyl strobate was accomplished by 40% $AgNO_3$-alumina column chromatography (3:7 ether-light petroleum) of methylated (CH_2N_2) cortex oleoresin. A pure material (99+% by GLC[13] on DEGS and on SE-30/EGiP) was obtained on crystallization from MeOH: m.p. 46·5–47°; $[\alpha]_D^{23}$ −48·9° (*c* 2·8, $CHCl_3$); mass spectrum *m/e* 316 (100%, M^+ for $C_{21}H_{32}O_2$ as determined from the high resolution spectrum), 301 (27%, M^+ —Me), 257 (33%, M^+ —COOMe), 241 (21%, M^+ —Me—COOMe) and 221 (25%, M^+ —C_7H_{11}); λ_{max} 193·6 nm (ϵ 15,400); NMR δ 5·44 (d, $J = 7$, one olefinic H), 5·41 (t, $J = 7$, one olefinic H), 3·63 (s, COOMe), 2·70 (m, one H at C-14), 1·66 (s, C-13 Me), 1·18 (s, C-4 Me), 1·17 (d, $J = 7$, C-14 Me), and 0·87 (s, C-10 Me), [Double resonance experiments show that the 2·70 H is coupled to both the 1·17 Me-(d) and the 5·44 olefinic H.]; $\nu_{max}^{CCl_4\text{-}CS_2}$ 1730 (C=O) and 1245 cm^{-1} (equatorial carbomethoxy); $\Delta\nu_{max}$ 1724 (C=O) and 1681, 1646 cm^{-1} (C=C); CD (*c* 0·013, isooctane) $[\theta]_{260}$ ±0°, $[\theta]_{230\cdot5}$ −4800°, $[\theta]_{225}$ ±0°, $[\theta]_{215}$ +22,000° (inflection), $[\theta]_{209\cdot5}$ +29,500°, $[\theta]_{203\cdot5}$ ±0°, $[\theta]_{193}$ −91,000°.

To facilitate a larger scale isolation of **1b**, the ether extract of eastern white pine cortex was extracted with 5% NaOH. This basic soln was acidified and extracted with ether and the ether soln methylated (CH_2N_2). The solvent was removed and the methylated product chromatographed (benzene) on neutral alumina activity III to yield 20 g of resin acid methyl esters which contained 27% methyl strobate. The esters were then hydrogenated in benzene with triphenylphosphine rhodium chloride for 18 hr at ambient conditions to selectively reduce the vinyl group of methyl pimarate, isopimarate, and sandaracopimarate; this simplifies the chromatographic purification, below, as strobate elutes between pimarate and isopimarate from $AgNO_3$-alumina.[9] After filtering through alumina and removing the benzene, the hydrogenated esters, in pentane, were filtered through a small bed of alumina. The 11·8 g of methyl esters thus obtained were chromatographed on 600 g of 40% $AgNO_3$-alumina with stepwise ester-light petroleum elution. A 1·9 g fraction of methyl strobate of 99+% purity was obtained.

Preparation of keto diester **2**

From methyl levopimarate. Keto diester **2** was prepared by exhaustive ozonolysis of methyl levopimarate as described by Pelletier.[10] The product was purified by chromatography on silica with 4:1 light petroleum-ether as eluant: $[\alpha]_D^{23}$ −50·7° (*c* 1·0, $CHCl_3$); NMR δ 3·72 and 3·68 (two COOMe singlets), and 1·20 and 0·76 (two Me singlets), lit.,[10] 3·71, 3·68, 1·22, and 0·76, respectively).

From methyl strobate. The keto diester was prepared as above: $[\alpha]_D^{25} -52{\cdot}4°$ (*c* 1·7, $CHCl_3$); NMR, IR, and GLC were identical with NMR, IR, and GLC of the keto diester from methyl levopimarate, above.

Preparation of the osmate ester of **3**

A soln of osmium tetroxide (89 mg, 0·35 mmole) in one ml pyridine was added to 102 mg (0·32 mmole) of **1b** in 10 ml ether. After 16 hr at room temp, the solvent was removed and the resulting black residue chromatographed on 10 g of silica (using acetone as eluant) to produce 221 mg (94% yield) of a brown-black solid. Recrystallization from 5% CH_2Cl_2 in heptane gave a crystalline product (homogeneous by TLC): m.p. 183–183·5°; NMR δ 8·92 (m, 4 aromatic H), 7·50 (m, 6 aromatic H), 5·00 (d, $J = 6$, one olefinic H), 4·52 (m, one H at C-12), 3·63 (s, COOMe), 3·40 (m, one H at C-14), 1·40 (s, Me at C-13), 1·23 (s, Me at C-4), 1·00 (d, $J = 7$, Me at C-14), and 0·95 (s, Me at C-10); ν_{max}^{KBr} 830(s), 1245, 1450(s), 1725 (C=O), and 3430 cm^{-1} (broad); λ_{max}^{EtOH} 263 (ε 7270), 257 (ε 8860), 252 (ε 8580), and 245 nm (ε 7730); CD (*c* 0·11, MeOH) $[\theta]_{487}$ −7600°; CD (*c* 0·13, CH_2Cl_2) $[\theta]_{503}$ −9270°.

Preparation of methyl 12α,13α-*dihydroxy*-14S,17-*cyclolabd*-8(17)-*en*-18-*oate* (**3**)

Osmium tetroxide (506 mg, 1·99 mmoles) dissolved in one ml pyridine was added to a soln of **1b** (569 mg, 1·80 mmoles) in 25 ml pyridine and allowed to react at room temp for 40 hr. A soln of 1·8 g $NaHSO_3$ in 40 ml 3 : 1 pyridine-water was then added with stirring until a clear red soln resulted[11] (*ca* 30 min). The chloroform extract (3 × 30 ml portions) of the red soln was washed with water, dried over $MgSO_4$, and the chloroform removed to yield 804 mg of a brown oil. Chromatography on 50 g of silica with 3 : 2 ether-light petroleum produced 443 mg clear oil which on crystallization from heptane gave 354 mg (56% yield, homogeneous by GLC and TLC) of white crystalline **3**: m.p. 142–143°; $[\alpha]_D^{25}$ +3·6° (*c* 1·8, $CHCl_3$); NMR δ 4·88 (m, one olefinic H), 3·62 (s, COOMe), 1·23 (s, Me at C-13), 1·05 (s, Me at C-4), 1·04 (d, $J = 7$, Me at C-14), and 0·89 (s, Me at C-10); $\nu_{max}^{CCl_4,CS_2}$ 1730, 1245, and 3540 cm^{-1} (broad, OH). (Found: C, 71·86; H, 9·72. $C_{21}H_{34}O_4$ requires: C, 72·00; H, 9·78%).

Preparation of methyl 12,15-*dioxo*-12,13-*secopimar*-8(14)-*en*-18-*oate** (**4**)

Diol **3** (31 mg, 0·09 mmole) was cleaved with sodium metaperiodate (0·26 mmole) as in the preparation of **6**, below. GLC showed the mixture contained 45% unreacted **3** and 55% product. Chromatography of the mixture on 3 g silica gave 15 mg **4** (homogeneous by TLC and GLC); NMR δ 9·80 (t, $J \cong 2$, CHO), 5·18 (d, $J = 10$, one olefinic H), 3·68 (COOMe), 2·13 (COMe), 1·17 (s, Me at C-4), 1·11 (d, $J = 7$, Me at C-13), and 0·82 (s, Me at C-10).

Preparation of methyl 12α,13α-*dihydroxy*-14S,17-*cyclolabdan*-18-*oate* (**5**)

A soln of 225 mg **3** in 20 ml EtOH containing 2 drops conc HCl and 40 mg Adams catalyst was hydrogenated for 20 hr at ambient conditions. On removing the catalyst and evaporating the solvent, 252 mg oil was obtained. Chromatography of the oil on 20 g of silica (3 : 7 ether-light petroleum) produced a 150 mg fraction which crystallized from heptane: m.p. 42–43·5°; $[\alpha]_D^{25}$ +28·2° (*c* 1·6, $CHCl_3$); NMR δ 3·62 (COOMe), 1·13 (s, Me at C-13), 1·07 (s, Me at C-4), 0·92 (d, $J = 7$, Me at C-14), and 0·80 (s, Me at C-10); $\nu_{max}^{CCl_4,CS_2}$ 1730 (C=O) and 3470 cm^{-1} (broad, OH); λ_{max}^{EtOH} 200 nm (ε 420). (Found: C, 71·42; H, 10·42. $C_{21}H_{36}O_4$ requires: C, 71·55; H, 10·30).

Preparation of methyl 12,15-*dioxo*-12,13-*secopimaran*-18-*oate* (**6**)

To a soln of 100 mg (0·28 mmole) of **5** in 5 ml of t-BuOH was added a soln of sodium metaperiodate (362 mg, 1·7 mmoles) in 10 ml of 1 : 1 t-BuOH-water. After stirring for 20 hr, the resulting soln was extracted with three 20 ml portions of ether. The combined ether extracts were washed with water, dried over $MgSO_4$, and the ether evaporated yielding 103 mg clear oil. This oil was chromatographed on 3 g of silica (1 : 4 ether-light petroleum eluant) to give 100 mg (98% yield) of **6** (homogeneous by GLC and TLC); $[\alpha]_D^{25}$ +20·6° (*c* 1·8, $CHCl_3$); NMR δ 9·76 (t, $J = 1{\cdot}5$, CHO), 3·64 (s, COOMe), 2·09 (s, COMe), 1·16 (s, Me at C-4), 0·99 (d, $J = 7$, Me at C-13), and 0·84 (s, Me at C-10); ν_{max}^{film} 1725 (C=O), 1245, and 2715 cm^{-1} (CHO). (Found: C, 72·27; H, 9·96. $C_{21}H_{34}O_4$ requires: C, 72·00; H, 9·78%).

Preparation of dimethyl 15-*oxo*-12,13-*secopimaran*-12,18-*dioate* (**7b**)

Jones reagent was added dropwise to a cooled soln of 62 mg **6** in 5 ml of acetone until a persistent orange color remained. After standing for 15 min, 15 ml water was added and the resulting soln extracted 3 times with 10 ml portions ether; the combined ether extracts were washed once with water and dried over Na_2SO_4. The ether was then removed yielding 66 mg clear oil which was separated into acidic and neutral fractions on one g of DEAE-Sephadex.[12] Crystallization of the acidic fraction (21 mg, 34% yield) from heptane gave **7a**, m.p. 106–108°. **7a** was methylated with ethereal diazomethane to produce **7b** as a clear oil (homogeneous by GLC and TLC): $[\alpha]_D^{25}$ +19·2° (*c* 2·0, $CHCl_3$); NMR δ 3·63 (s, two COOMe), 2·08 (s, COMe), 1·15 (s, Me at C-4), 0·99 (d, $J = 7$, Me at C-13), and 0·81 (s, Me at C-10); ν_{max}^{film} 1730 (C=O) and 1245 cm^{-1}; CD (*c* 0·13, MeOH) $[\theta]_{283}$ +3660°; CD (*c* 0·27, hexane) $[\theta]_{330}$ ±0°, $[\theta]_{312}$ +1080° (inflection), $[\theta]_{302}$ +2410°, $[\theta]_{299}$ +2380°, $[\theta]_{292}$ +2980°, $[\theta]_{287}$ +2780°, $[\theta]_{285}$ +2800°, and $[\theta]_{250}$ +295°. (Found: C, 69·63; H, 9·63. $C_{22}H_{36}O_5$ requires: C, 69·50; H, 9·54%).

Hydrogenation of methyl strobate. Hydrogenation of 200 mg of methyl strobate in 30 ml of abs EtOH for 18 hr at ambient conditions in the presence of 800 mg of catalyst (5% Pd-C) gave 4 major products. Chromatography of the hydrogenation mixture on 100 g $AgNO_3$-alumina with stepwise ether-light petroleum elution gave a fraction consisting of mixed tetrahydro derivatives and, subsequently, pure fractions of two dihydro derivatives. The first of the dihydrostrobates to elute was **8** which crystallized from MeOH: m.p. 105–107°; $[\alpha]_D^{25}$ −80·0° (*c* 1·3, $CHCl_3$); NMR δ 3·63 (s, COOMe), 1·57 and 1·60 (s, two Me's on double bond), 1·17 (s, Me at C-4), and 0·99 (s, Me at C-10); ν_{max}^{film} 1730 and 1245 cm^{-1}; λ_{max} 195 nm (ε 8760); CD (*c* 0·01), isooctane) $[\theta]_{260}$ ±0°, $[\theta]_{197{\cdot}5}$ −34,500°, $[\theta]_{190}$ −29,600°. The second dihydrostrobate, **9** eluted as an oil: $[\alpha]_D^{25}$ −8·6° (*c* 2·4, $CHCl_3$); NMR δ 4·99 (d, $J = 6$, one olefinic H), 3·62 (s, COOMe), 1·22 (s, Me at C-4), 0·98 (d, $J = 6$, Me at C-14), 0·88 (s, Me at

*The ring-cleaved products, **4**, **6**, and **7** are correctly named as "secopimaranes" rather than "seco-cyclolabdanes," see Ref 2.

Table 1. GLC and TLC data for methyl strobate oxidation products

Compound	GLC[a] r_{pim}	TLC[b] R_f
1b	1·43	0·95
3	3·93	0·30
4	3·05	0·57
5	4·56	0·49
6	3·31	0·60
7	3·64	0·57

[a]Retention time relative to methyl pimarate for a 3% SE-30 on 70/80 Anakrom ABS column, 6 ft × 1/8 in, at 200° and F'_A of 50 ml/min; Hewlett-Packard 5750 instrument.

[b]Silica Gel IB-F plates (Baker Chemical Co.) developed with 3:2 ether-pentane.

Table 2. GLC retention data for methyl strobate and derivatives

Compound	r_{pim}[a] DEGS	SE-30/EGiP
Methyl strobate (**1b**)	1·74	1·39
dihydrostrobate (**8**)	1·93	1·71
dihydrostrobate (**9**)	1·39	1·31
tetrahydrostrobate (**10**)	1·03	1·25
tetrahydrostrobate (**11**)	1·39	1·56
keto diester (**2**)	8·03	1·00

[a]r_{pim} = Retention relative to methyl pimarate; for GLC conditions see Ref 13.

C-10), and 0·75 (d, $J = 7$, Me at C-13); ν_{max}^{film} 1732, 1245, and 852 cm^{-1}; mass spectrum *m/e* 318·2541 (318·2557 calc., 17%, M$^+$), 303 (4%, M$^+$ —Me), 259 (17%, M$^+$ —COOMe), 243 (4%, M$^+$ —Me—COOMe), 221 (2%, M$^+$ —C_7H_{13}), and 121 (100%, M$^+$ —$C_{12}H_{21}O_2$); λ_{max} 203·2 nm (ε 9600); CD* (*c* 0·036, isooctane) $[\theta]_{260}$ ±0°, $[\theta]_{221}$ +4750°, (*c* 0·012) $[\theta]_{207}$ ±0°, $[\theta]_{195}$ −11,700°, $[\theta]_{192}$ −11,300°.

The tetrahydro derivatives were separated by preparative GLC on 20% DEGS to yield two pure fractions. Crystallization of the first fraction from MeOH gave **10**: m.p. 68·0–68·5°; NMR δ 3·62 (s, COOMe), 1·13 (s, Me at C-4), *ca* 0·88 (m, two secondary Me's), and 0·80 (s, Me at C-10); ν_{max}^{film} 1730 and 1245 cm^{-1}; mass spectrum *m/e* 320·2714 (calc. 320·2714, 80%, M$^+$), 261 (58%, M$^+$ —COOMe), 245 (10%, M$^+$—Me—COOMe), 163 (100%, M$^+$ —$C_9H_{17}O_2$); $[\alpha]_D^{25}$ +8·1° (*c* 1·6, $CHCl_3$). Crystallization of the second tetrahydrostrobate from MeOH gave **11**: m.p. 72–73·5°; NMR δ 3·62 (s, COOMe), 1·17 (s, Me at C-4), 0·96 (s, Me at C-10), and 0·79 and 0·80 (d, two Me's); $\nu_{max}^{CCl_4,CS_2}$ 1730 and 1245 cm^{-1}; $[\alpha]_D^{25}$ −12·1° (*c* 1·1, $CHCl_3$).

*For comparison, the CD of several related monoenoates were also determined in isooctane: Methyl 8(14)-pimaren-18-oate (*c* 0·011) $[\theta]_{260}$ ±0°, $[\theta]_{207}$ +32,000°, $[\theta]_{198}$ +8600°; methyl 8(14)-isopimaren-18-oate (c 0·013) $[\theta]_{260}$ ±0°, $[\theta]_{206}$ +25,700°, $[\theta]_{195}$ +7000°; methyl 7-isopimaren-18-oate (*c* 0·01) $[\theta]$ ±0°, $[\theta]_{207}$ −28,200°, $[\theta]_{196}$ −18,700°, $[\theta]_{192.5}$ −22,800°; methyl 13β-abiet-8(14)-en-18-oate (*c* 0·011) $[\theta]_{260}$ ±0°, $[\theta]_{207.5}$ +17,700° (lit.[6] for acid, $[\theta]_{209}$ +17,000°), $[\theta]_{197}$ +6600°. *cf.* Ref. 6 for CD data for other abietenoates.

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