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HIGHER ISOPRENOIDS—VIII[1]

DITERPENOIDS FROM THE OLEORESIN OF *HARDWICKIA PINNATA* PART 1: HARDWICKIIC ACID†

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Abstract—The oleoresin from *Hardwickia pinnata* Roxb. is shown to be a complex blend of sesquiterpenes and diterpenes. (−)-Copaene, (−)-caryophyllene and humulene account for over 90% of the sesquiterpenes present. The diterpenes, which constitute some 60% of the oleoresin, have been found to consist of a series of closely related new diterpenoids of *ent*-clerodane type. Evidence leading to the elucidation of the absolute stereostructure of the main diterpenoid—hardwickiic acid—is presented.

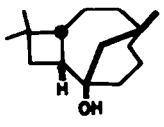
Hardwickia pinnata Roxb. syn. *Kingiodendron pinnatum* (Roxb.) Harms (Malayalam, Tamil: *Kolavu*) is a large handsome tree growing wildly in the evergreen forests of Western Ghats of India. The tree, on tapping, yields in large quantities, a dark or reddish brown oleoresin (balsam).[2] Though, the oleoresin has been subject of a few previous investigations,[3,4] these were restricted to the steam-volatile components only. The present investigations, which were primarily aimed at the higher terpene components, have shed additional light on the composition of the essential oil and have led to the isolation seven new diterpenoids from the resin part. The present communication describes the isolation/identification of these sesquiterpene and diterpene components and unfolds evidence leading to the absolute stereostructure of the main diterpenoid, an acid, which we name hardwickiic acid.

The oleoresin was segregated into acidic (~60%) and neutral (~40%) fractions and the latter further separated into steam-volatile (90%; essential oil) and steam-non-volatile portions.

By judicious combination of glc,* tlc over SiO_2 gel–$AgNO_3$,[5] column chromatography over SiO_2 gel–$AgNO_3$, most of the components of essential oil were separated and identified by usual comparison with authentic samples,[6] and the final essential oil composition was found to be: (in order of increasing GLC retention time) (−)-copaene (4.5%), (−)-caryophyllene (75%), humulene (13%), (−)-carophyllene oxide (2.5)%, caryophyllene alcohol (1%), humulene oxide-I (0.5%) humulene oxide-II (0.5%), caryophyllenol-I and caryophyllenol-II (0.2%), besides at least six unidentified components (amount to ~3%). The isolation of caryophyllene alcohol (1)[7] is considered significant, as it is an acid-catalyzed hydration product of caryophyllene (2).

It is conceivable that this alcohol is a product of a non-enzymatic reaction, as the exudate from the tree is rich in acids and caryophyllene, and contains some water. A survey of the literature showed that caryophyllene alcohol had been previously isolated from *Mentha arvenis*[8] and *Mentha piperata*,[9] but since its isolation from these sources entailed treatment with boric acid and the oils also contain caryophyllene, it was, in all probability, an artifact.

Likewise, from the steam-non-volatile neutral fraction, besides β-sitosterol and clovane-diol[10] (3), two new diterpene alcohols and a diterpene methyl ester (Table 1) were isolated.



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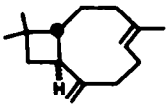

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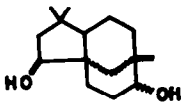

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A preliminary separation of the acids was first carried out on the basis of solubility characteristics of the sodium salts, and then the appropriate fractions further segregated and resolved into pure components *via* cyclohexylamine salt formation, suitable solvent extraction or chromotography of the derived methyl esters. In this way, five new diterpene acids could be isolated. Table 1 lists these compounds, along with some of their characteristics and approximate relative percentages, which are based on glc.

Structures of all these compounds have been elucidated in complete stereochemical detail and the major results reported in two preliminary communications.[11,12] The present paper reports details of these investigation for the major diterpene acid—hardwickiic acid; subsequent communications will describe details pertaining to remaining diterpenoids.

Hardwickiic acid. Hardwickiic acid*, $C_{20}H_{28}O_3$, is clearly a monobasic carboxylic acid: IR, 1681 cm^{-1} (dimeric carboxyl function); cyclohexylamine salt (m.p. 143–144°); methyl ester ($C_{21}H_{30}O_3$, M^+, *m/e* 330; IR: COOMe 1715 cm^{-1}). It has no hydroxyl function (IR, Me ester) and the presence of a trisubstituted olefinic linkage (IR: 1629, 814 cm^{-1}. PMR: 1 H, t, 6.77 ppm, J = 4 Hz) and a β-substituted furan ring (+ ve Ehrlich test.[13] IR:[14]

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*Since the publication of preliminary communications,[11,12] (−)-hardwickiic acid has been found to occur in *Gossweilerodendron balsamiferum* Harms[16] and *Croton oblongifolius*.[17] Its antipode has been isolated from *Copaifera officinalis*[18] and *Ribes nigrum* L.[19]

Table 1. Diterpenoids from the oleoresin of *Hardwickia pinnata* Roxb.

No.	Trivial name	R_{dye}*	RRT+	Mol. formula	m.p.	n_D^{30}	$[\alpha]_D$‡	Approx. relative % (GLC)
				Alcohols				
1	Kolavenol†	0.70	-	$C_{20}H_{34}O$	-	1.5166	-42.0°	-
2	Kolavelool†	0.78	-	$C_{20}H_{34}O$	-	1.5108	-30.8°	-
				Acids/Me esters				
3	Kolavonic acid† (Me ester)	0.74	0.70	$C_{19}H_{30}O_3$	-	1.5114	-144.6°	5
4	Kolavenic acid† (Me ester)	1.13	0.83	$C_{21}H_{34}O_2$	-	1.5136	-65.6°	20
5	Kolavenolic acid† (Me ester)	-	-	$C_{21}H_{36}O_3$	95-96°	-	-45.0°	?
6	Hardwickiic acid	1.10	1.00	$C_{21}H_{28}O_3$	106-107°	-	-133.9°	50
7	Kolavic acid†	0.84	1.60	$C_{20}H_{30}O_4$	228-230°	-	-115.0°	7

* TLC, R_s with respect to Sudan III: silica gel G, 0.3 mm layer; solvent system, 20% EtOAc in C_6H_6 (for alcohols), C_6H_6 (for methyl esters of acids).

+ GLC, relative retention time of Me esters: 20% silicone SE-30 on 60-80 mesh Chromosorb W: column, 150 cm x 5 mm; temp. 250°: gas flow, 50 ml H_2/min.

‡ Solvent, $CHCl_3$ except for kolavic acid, for which EtOH was used.

† Names derived from the local name (kolavu) of the tree.

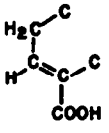
1560, 1500, 877 cm^{-1}. PMR:[15] β'-proton, 1 H, m, 6.12 ppm; α-proton, 1 H, bs, 7.05 ppm; α'-proton, 1 H, t, 7.20 ppm, J = 1.5 Hz) are clearly indicated. From its PMR spectrum, the presence of two tertiary methyls (3 H singlets at 0.80 and 1.27 ppm) and one CH_3–CH (3 H, d, 0.85 ppm, J = 6 Hz) is also evident.

The conjugation of the COOH and the olefinic function is inferred from the position of the C=O absorption and the intensity of the C=C stretching in the IR spectra of the acid and its Me ester, as well as from the UV absorption (Me ester, λ_{max} 213 nm, ϵ 12560; summation of furan[20] and $\alpha\beta$-unsaturated carboxyl ester[21] absorptions). This is further borne out from the expected upfield shift of the C=CH absorption in the PMR spectra in going from the acid (6.77 ppm) or its Me ester (6.57 ppm) to the derived (LAH reduction) alcohol (hardwickiol, 5.43 ppm).

From the above data, besides the presence of β-substituted furan moiety, part structure 4 can be concluded, keeping in mind the multiplicity of the C=CH signal.

Methyl hardwickiate, on quantitative hydrogenation over 5% Rh–C or Rh–Pt absorbed two mole equivalents of hydrogen to furnish a tetrahydroderivative, $C_{21}H_{34}O_3$, in which the function 4 was still in tact: UV (λ_{max} 215 nm, ϵ 6960), IR (C=O 1715 cm^{-1}; C=C 1635 cm^{-1}), PMR (1 H, t, 6.5 ppm, J = 4 Hz). Curtius–Oesterlin degradation[22] of tetrahydrohardwickiic acid furnished, as expected, a ketone, which from its carbonyl frequency (1718 cm^{-1}) is clearly 6-membered. Thus, part structure 4 can be expanded to 5.

On further hydrogenation over PtO_2 catalyst, methyl tetrahydro-hardwickiate absorbed one mole equivalent of H_2 to furnish a hexahydroderivative, which was devoid of any olefinic unsaturation (UV, IR, PMR and tetranitromethane test). Thus, it is clear that hardwickiic acid has a total of three C=C linkages and being $C_{20}H_{28}O_3$ and incorporating a cyclic ether (furan) and carboxyl function, must be bicarbocyclic.



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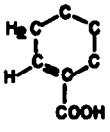

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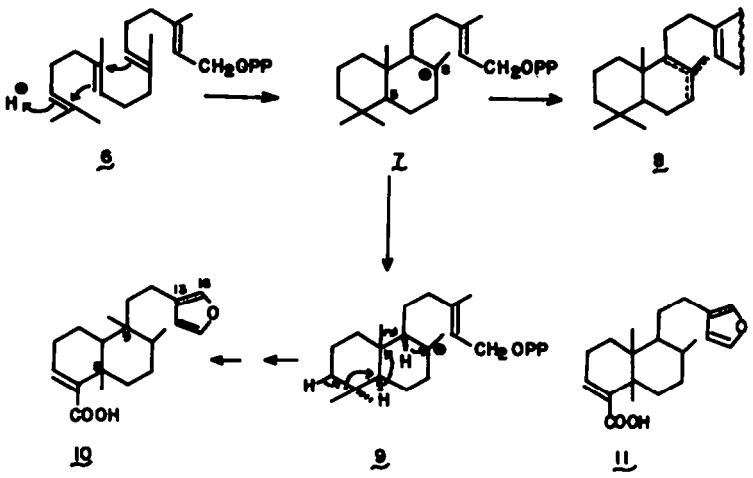
On catalytic dehydrogenation[23] over 10% Pd–C, hardwickiic acid gave, besides other non-aromatic products, an approximately 8% yield (on weight basis) of a 3:2 (glc) mixture of 1,2-dimethyl and 1,2,5-trimethylnaphthalenes.

From the number of Me groups (free and "functionalised") it is clear that hardwickiic acid is a bicarbocyclic diterpenoid. While looking for a suitable framework for the various structural features, it became clear that both, a secondary Me and an α,β-unsaturated carboxyl function, *cannot* be accommodated in a normal bicyclic diterpene framework (8) with furan in the side-chain. This dictates a rearranged bicyclic skeleton for the new acid and, gross structure 10, which can be generated from the ion 7 (Fig. 1), the accepted[24] biogenetic progenitor for many bicyclic diterpene types, appeared most appropriate, as it not only meets all the structural requirements of hardwickiic acid, but would also account for the preferential formation of 1,2-dimethylnaphthalene during dehydrogenation. Another possibility (11), which can also be accommodated[b] in the Biogenetic Isoprene Rule, is considered less likely in view of the dehydrogenation results.

To prove unequivocally, the gross structure 10, it is

[b] Reduction of 7 at C-8, followed by incorporation of a suitable leaving group (e.g. OX) at C-5 can generate a suitable precursor for 11. *cf* e.g. diterpene alkaloid, thelepogine.[25]



6 7 8 10 9 11

Fig. 1.

essential to adduce sound evidence for (i) presence of Me groups at C-5 and C-9, and (ii) relative disposition of the functionalities. With this objective in view, as also to elucidate absolute stereochemistry at the various chiral centres, suitable degradation studies were, next, carried out and, the results leading to the establishment of absolute stereostructure 12 for (−)-hardwickiic acid are summarised below.

Evidence for the location of tertiary methyls at C-5 *and* C-9. Ozonolysis of hardwickiic acid, followed by oxidative (H_2O_2) work-up of the ozonide, yielded a complex mixture of at least six (tlc) products, from which two major and one minor component (as methyl esters) could be obtained pure. The analytical and spectroscopic data (IR, PMR; Table 2) of these compounds are entirely consistent with the respective structures 13, 14 and 15 (Table 2), derivable from structure 10 for

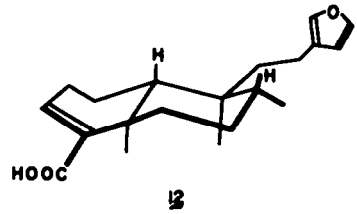


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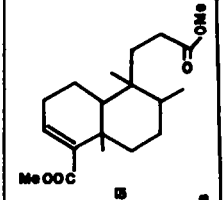
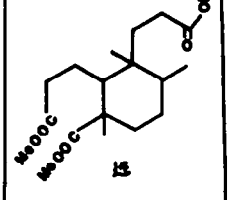
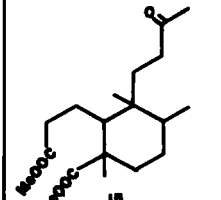
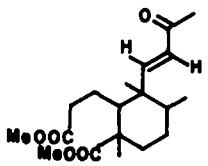
Table 2. Products (Me esters) of ozononolysis of hardwickiic acid

Product	Yield (wt. basis)	EtOH λ_{max} (nm)	ν(C=O) (cm^{-1})	δ (ppm)			
				Me-C-	Me-CH (d, J= 6Hz)	COOMe	Others
 13	3%	220 (ε, 4050)	1720 1750	0.75 1.20	0.82	3.60 3.60	6.57 (C=CH, t, J=4Hz)
 14	33%	-	1754	0.83 1.20	0.85	3.65 3.65 3.65	-
 15	21%	-	1735 1750	0.82 1.18	0.86	3.65 3.65	2.13 (MeCO)

hardwickiic acid. As expected, NaOBr oxidation of **15** furnished, after esterification, **14**. The formation of **15** during ozonolysis-oxidative work-up of **10** (hardwickiic acid) deserves a brief comment. Evidently, **15** arises from partial ozonolysis of furan ring at C(13)=C(16), leading to a β-keto aldehyde (after hydrolytic loss of HCOOH), which by a retro-aldol fragmentation generates **15**.

The PMR spectra of both **14** and **15** show (Table 2) one of the tertiary Me's downfield (at δ 1.20 and 1.18 ppm respectively) as required[26] for a Me group α to a carbomethoxy function, thus supporting the presence of a tertiary methyl at C-5 in **10**. This conclusion is reinforced by comparison of the δ values for the two tertiary Me's in different derivatives of hardwickiic acid (Table 3). The upfield shift observed for one of the tertiary Me in going from methyl hardwickiate to its hexahydroderivative (loss of olefinic bond) or to hexahydrohardwickiol (loss of olefinic bond and reduction of COOMe to CH_2OH) is fully consistent with that Me being on the carbon α to the olefinic linkage,[27] as is the case with structure **10** (hardwickiic acid).

Compound **15** came handy in locating the position of the second tertiary Me. Bromination of **15** with NBS or with Br_2–Et_2O complex,[28] furnished a bromo derivative, which on dehydrohalogenation with $CaCO_3$–DMF,[29] gave an α,β-unsaturated ketone: λ_{max} 227 nm (ϵ 12,850); IR, C=C–C=O 1695, 1645 cm^{-1}. Its PMR spectrum shows two olefinic protons as an AB-quartet centred at 6.33 ppm, J_{AB} = 16 Hz, $J_{AB}/\delta_B - \delta_A) = 0.47$, a finding consistent only with fully substituted C(9), as shown in **16**. This placement of the tertiary Me at C(9) is further supported by the position of its PMR signal (in **16**), which as required[30] by its being on an allylic carbon has now suffered a downfield shift (0.95 ppm as compared to 0.83 and 0.82 ppm in **14** and **15** respectively; the other tertiary Me signal remains essentially unchanged at 1.21 ppm).



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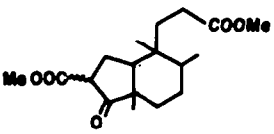
The data obtained so far suffice for confirmation of the gross structure **10** for hardwickiic acid. Transformations described below not only helped to establish the absolute stereochemistry at each of the chiral centre, but also further corroborated the above conclusions.

Ring junction. The triester **14** on Dieckmann cyclization (NaH) gave, as expected, the cyclopentanone carboxylate ester **17** (epimeric mixture), which on acid-hydrolysis furnished the required single cyclopentanone derivative **18** (IR, 1740 cm^{-1}). This ketone shows in its CD curve, a strong negative Cotton effect ($\Delta\epsilon_{301} - 2.49$, τ = 36 nm; dioxane, Fig. 2), which is almost a mirror image (except for reduced intensity) of that observed for a 17-keto-steroid, e.g. 3β - hydroxy - 5α - androstan - 17 - one (**19**) ($\Delta\epsilon_{300} + 3.25$, Γ = 34 nm; dioxane).[31] This implies that the two rings in **18** must be *trans*-locked, like the C/D rings of an androstan-17-one, but with opposite absolute stereochemistry, as shown in **20**.



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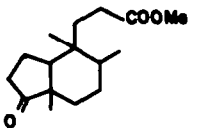

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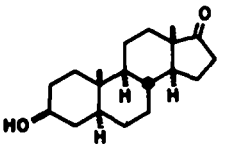

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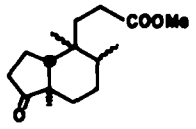

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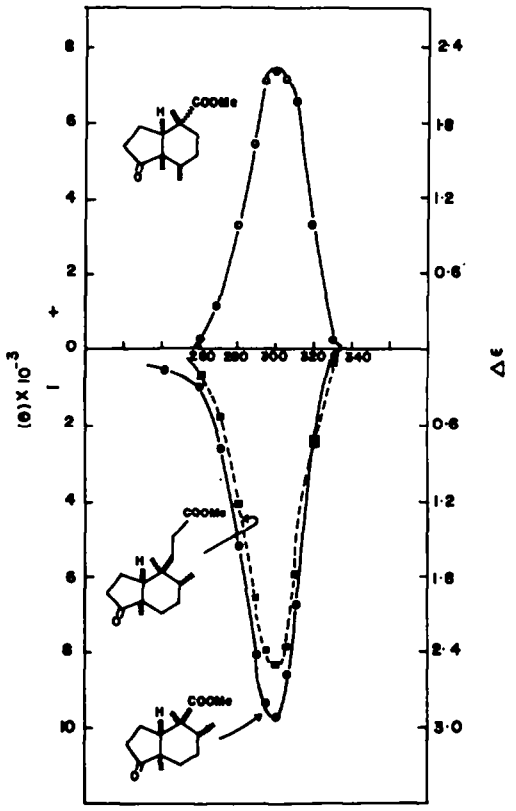


Fig. 2.

Configuration at C(9). To obtain information about the configuration at C(9), the keto ester **20** was suitably degraded (Fig. 3) to the bis-nor keto ester **24**, by employing modified version of Barbier–Wieland degradation, which was reported some years ago.[32] RuO_4 oxidation[33] was found to be more convenient and expeditious, than CrO_3 oxidation. From a comparison of the tertiary Me resonances (tertiary Me's at C-5 and C-9 respectively; the assignments are obvious from the respective structures) for the bisnor keto estor **24** (0.92, 1.07) and the related compounds **14** (1.20, 0.83), **20** (0.97, 0.83) and **22** (0.90, 0.78), it is clear that the carbomethhoxy group at C(9) in **24** has little influence on the C(5)–Me and hence, it must be β-oriented as shown in **25**, as in the alternative α-configuration, it will have 1,3-diaxial relationship with the C(5)–Me which will then be subject to the shielding

Table 3. PMR Spectral (CCl_4) data of hardwickiic acid and its derivatives

No.	Compound	δ(ppm)							
		Me-C- (s)	Me-C- (s)	Me-CH (d, J≈6Hz)	C=CH (t, J≈4Hz)	furan-αH	furan-α'H (t, J≈1.5Hz)	furan-β'H	Others
1	Hardwickiic acid	1.27	0.80	0.85	6.77	7.05(bs)	7.20	6.12(m)	11.65(bs, COOH)
2	Methyl hardwickiate	1.27	0.80	0.85	6.57	7.17(bs)	7.32	6.17(m)	3.65(s, COOMe)
3	Methyl tetrahydro-hardwickiate	1.22	0.73	0.80	6.50	-	-	-	3.62(s, COOMe) 3.05-3.90 (m, CH_2OCH_2)
4	Methyl hexahydro-hardwickiate	0.98	0.70	0.77	-	-	-	-	3.55(s, COOMe) 3.03-3.87 (m, CH_2OCH_2)
5	Hardwickiol	1.06	0.75	0.77	5.43	7.07(bs)	7.20	6.10(m)	3.95(bs, CH_2OH)
6	Hardwickiol acetate	1.05	0.73	0.80	5.47	7.05(m)	7.18	6.10(m)	4.40(bs, CH_2OAc) 1.95(s, MeCO)
7	Hexahydrohardwickiol	0.83	0.70	0.79	-	-	-	-	3.05-3.80 (6H, CH_2OH, CH_2OCH_2)

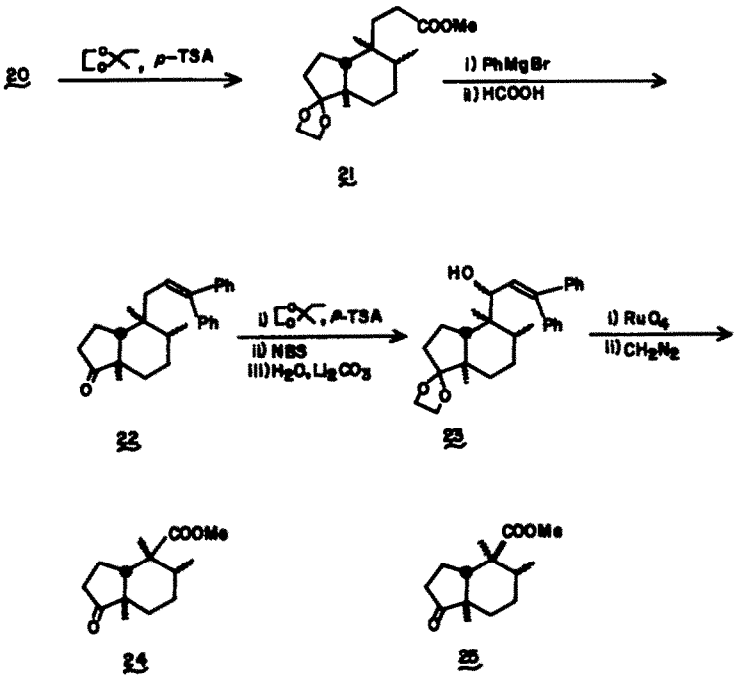
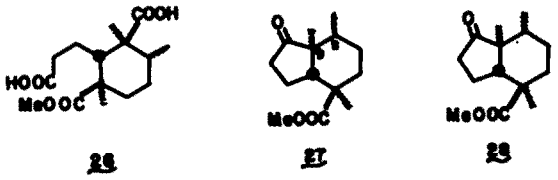


Fig. 3.

cone of the carbonyl group.[34] This conclusion was reinforced from CD of ketone **27** described below.

As expected, **25**, like its parent ester **20**, shows a strong negative Cotton effect in its CD curve: $\Delta\epsilon_{302} - 2.91$, $\Gamma = 37$ nm (dioxane) (Fig. 2).

Configuration at C(8). The unsaturated keto diester **16** was oxidised with RuO_4 and the product hydrolysed with 1% KOH–MeOH (1 hr) to furnish the monoester dicarboxylic acid, which in view of our previous conclusions, can be formulated as **26**. This, on being refluxed with Ac_2O, followed by distillation[35] at ~200°/2 mm, gave the



expected (Blanc rule[36]) cyclopentanone **27**: IR, C=O 1740 cm^{-1}. Its CD shows a positive Cotton effect ($\Delta\epsilon_{298-306} + 2.18$, $\Gamma = 38$ nm, dioxane; Fig. 2) as, indeed is required for the absolute configuration of ring-junction shown in **27**; this provides further evidence for C(9)-chirality, as already deduced above. The PMR spectrum of **27** shows the C(8)–Me resonance as a doublet centred at 1.19 ppm (J = 7 Hz). In all other compounds described so far, the C(8)–Me doublet is usually centred at ~0.83 ppm (see Tables 2 and 3). This large paramagnetic shift in **27** must have been caused by the keto group, with which it has a "peri" relationship. Since, a CO group will deshield only those protons which lie in its plane, the C(8)–Me must be equatorial, as shown in **28**.

The above experiments, thus, enable us to uniquely describe the absolute stereostrcctureᶜ of (−)-hardwickiic acid as **12**. (−)-Hardwickiic acid, thus, became the first simple member of the rearranged labdanes, represented at that time[45] by such highly oxygenated compounds as clerodin, cascarillin and columbin. It was also the first diterpene with *ent*-clerodane absolute stereochemistry.

ᶜSince the publication of the preliminary communication[12] on absolute stereochemistry, (−)-hardwickiic acid has served as the central reference compound for interrelating the absolute stereochemistry of a number of other naturally occurring diterpenoids.[17,18,37–44]

EXPERIMENTAL

All m.ps and b.ps are uncorrected. Light petroleum refers to fraction b.p. 40–60°. All solvent extracts were finally washed with brine, before drying (Na_2SO_4). Rotations were measured in $CHCl_3$ soln, unless otherwise stated.

IR spectra were taken on a Perkin–Elmer Infracord model 137E, either as smears (liquids) or in Nujol (solids). PMR spectra were recorded for a ~10% soln in CCl_4 with TMS as the internal standard, on a Varian Associates A-60 spectrometer. Glc was carried out on Aerograph, model A-350-B, using 150 cm × 5 mm Al column packed with 20% Silicone SE-30 on 60–80 mesh Chromosorb W, unless stated to the contrary.

Alumina employed in this work was washed with nitric acid[46] and activated at 460° for 6 hr and graded according to Brockmann.[47] Tlc was carried out on 0.3 mm silica gel (containing 15% plaster of Paris) layers.

The oleoresin. The material was collected from a 40 year old tree located at Choodal side in Kulathupuzha Reserve in Thenmale range, during the month of January, through the courtesy of the Divisional Forest Officer, Thenmala.

Separation into main groups. The oleoresin (200 g) was dissolved in 400 ml of light petroleum (and filtered to remove any extraneous matter) and shaken with 10% NaOH aq (200 ml, 100 ml). Three distinct layers were formed each time; aq. layer (soluble Na salts), semi-solid interphase (essentially sparingly soluble Na salts), and light petroleum layer. The three phases were separated and pooled.

The semi-solid material was dissolved in water (500 ml), and extracted with 1:1 ether–hexane (100 ml × 2). This extract was added to the main light petroleum layer. The aq part was treated with brine (150 ml) and the precipitated sparingly soluble Na salts

(gummy) collected by filtration. The filtrate was mixed with the main aq. layer and extracted with light petroleum (100 ml + 1) and the extract added to the initial light petroleum layer. The combined solvent extracts were washed with water (50 ml × 4), brine (50 ml × 2) and dried. Solvent removal furnished neutral material (~90 g, ~45%). This, as well as, the soluble Na salts and sparingly soluble Na salts were appropriately treated as described below.

Neutral components. The neutral material (88 g) was steam-distilled by cohobation, using a set-up of a type generally employed for azeotropic distillation (removal of lighter phase),[48] to furnish an essential oil which was totally distilled: 73 g, b.p. 95–102°/2.5 mm, n_D^{30} 1.4950, α_D −8.7°. The steam non-volatile portion was taken up in ether, washed with brine, dried and freed of solvent to get a very viscous product (15 g).

The essential oil. Glc (20% diethylene glycol polysuccinate on Chromosorb W, 60–80 mesh/200 cm × 5 mm Al column; temp 16°; 50 ml H_2/min) revealed this material to consist of at least 15 components. The material (40 g) was fractionated on an efficient column into a hydrocarbon (b.p. 102–107°/4 mm, 36.5 g) and an oxygenated compound cut (b.p. 107–130°/1 mm, 2.7 g). Each of these cuts was further segregated on $AgNO_3$-silica gel, by following the usual procedure.[49] Both fractionation and chromatography were monitored by glc and $AgNO_3$-silica gel tlc.[5] The pure compounds (see text), thus obtained, were identified by comparison (IR, PMR, glc) with authentic samples.

Caryophyllene alcohol (1), m.p. 93–94° (acetonitrile), $[\alpha]_D$ −3.06° (lit.:[4] m.p. 95–96°).

Steam non-volatile material. This material (17.9 g) was chromatographed on Al_2O_3/II (26.5 cm × 5 cm) with tlc (solvent: 10% EtOAc in C_6H_6) monitoring.

Fraction 1	light pet.	100 ml × 20	3.95 g, hydrocarbon
	C_6H_6	100 ml × 20	mixture, rejected
Fraction 2	C_6H_6	100 ml × 24	0.81 g, impure kolavelool
Fraction 3	C_6H_6	100 ml × 24	0.82 g, impure methyl kolavenolate
Fraction 4	1% MeOH in C_6H_6	100 ml × 40	6.73 g, crude kolavenol
Fraction 5	2% MeOH in C_6H_6	100 ml × 40	0.74 g, crude β-sitosterol
Fraction 6	10% MeOH in C_6H_6	100 ml × 16	0.41 g, crude clovane-diol
Fraction 7	MeOH	100 ml × 30	0.13 g, mixture, rejected

Kolavelool. Pure kolavelool was isolated from fraction 2 by preparative layer chromatography (PLC) (1 mm silica gel layers) followed by hydrolysis with 5% KOH aq–EtOH (2 hr, reflux) to remove an ester impurity, followed by another PLC: viscous liquid (63 mg), b.p. 110–120° (bath)/4.33×10^{-3} mm, n_D^{30} 1.5108, $[\alpha]_D^{30}$ −30.8° (*c*, 0.53%). (Found: C, 83.09; H, 11.81. $C_{20}H_{34}O$ requires: C, 82.69; H, 11.80%).

Methyl kolavenolate. Fraction 3 on repeated chromatography over SiO_2 gel/III, furnished besides a somewhat impure (tlc, PMR) sample of a possibly new diterpene alcohol,[4] a crystalline hydroxy ester, named methyl kolavenolate: colourless rods (CH_3CN), m.p. 95–96°, $[\alpha]_D$ −45.0° (*c*, 0.87%). (Found: C, 75.28; H, 10.90. $C_{21}H_{36}O_3$ requires: C, 74.95; H, 10.78%).

Kolavenol. Fraction 4 on rechromatography (SiO_2 gel/III) furnished a pure diterpene alcohol, which we name kolavenol: colourless viscous liquid, b.p. 150–160° (bath)/7.7×10^{-3} mm, n_D^{30} 1.5166, $[\alpha]_D^{30}$ −42.0° (*c*, 3.48%). (Found: C, 82.52; H, 11.70. $C_{20}H_{34}O$ requires: C, 82.69; H, 11.80%).

β-Sitosterol from fraction 5: m.p. 138–139° (MeCN), $[\alpha]_D^{27}$ −34.3° (*c*, 1.44%). Identified by comparison (tlc, IR, PMR) with an authentic sample.

Clovane-diol (3). Fraction 6 on recrystallization from MeCN, furnished colourless needles, m.p. 155–156°. (Found: C, 75.68; H, 11.02. $C_{15}H_{26}O_2$ requires: C, 75.58; H, 11.00%). Identified by comparison (IR, PMR, m.m.p.) with an authentic sample.[48]

[4]The spectral characteristics (UV, IR, PMR) of this material (liquid) clearly reveal it to be an impure specimen of agbaninol, later isolated by Ekong and Okogun[36] from the African timber *Gossweilerodendron balsamiferum*.

Acids from soluble sodium salts

Kolavic acid. The combined aq alkaline layer (containing soluble Na salts) on acidification (conc HCl aq) at 0°, gave a dirty white gelatinous ppt, which was collected and dried, m.p. 102–210°, yield 6.78 g. This material (5.0 g) was triturated with dry CCl_4 (25 ml) at room temp. (~25°) and the soluble part removed by inverse filtration; this treatment was repeated once more. The CCl_4-insoluble material (~85%), on further trituration with cold dry ether (10 ml × 2) yielded a product (3.1 g), m.p. 213–222°. This on repeated crystallization from MeOH furnished lustrous rods (2.5 g) of a new diterpene acid, named kolavic acid: m.p. 228–230°, $[\alpha]_D^{27}$ −115.3° (*c*, 1.8% in EtOH). (Found: C, 72.06; H, 8.87. $C_{20}H_{30}O_4$ requires: C, 71.82; H, 9.04%).

Kolavonic acid. The CCl_4-soluble, as well as ether-soluble portions from the above were complex mixtures (tlc, glc of Me esters, see Table 1), in which kolavic acid still predominated. The ether-soluble part (m.p. 95–120°) was esterified (CH_2N_2) and the product (6.0 g, from a larger separation) chromatographed over Al_2O_3/II (26.0 cm × 2.5 cm) with tlc monitoring (solvent: 5% EtOAc in C_6H_6) with a view to isolate any minor component. Light pet, light pet-benzene mixture (1:1), benzene and then, benzene containing increasing quantities of MeOH (1 to 5%) were used one after the other in the usual manner. Light pet–C_6H_6 (50 ml × 10) and C_6H_6 (50 ml × 3), together yielded 2.8 g of methyl kolavate. Later C_6H_6 fractions yielded 46 mg of another pure ester (tlc, glc, see Table 1), now named (methylester of) kolavonic acid: viscous liquid, b.p. 125–135° (bath)/1.92×10^{-3} mm, n_D^{30} 1.5114, $[\alpha]_D^{27}$ −114.6° (c, 0.71%). (Found: C, 74.15; H, 9.37. $C_{19}H_{30}O_3$ requires: C, 74.47; H, 9.87%).

Acids from sparingly soluble sodium salts

The semi-solid Na salts (from 200 g of the oleoresin) were dissolved in water (500 ml), acidified with conc HCl aq (Congo red) and the product taken up in ether (100 ml × 4). The combined ether extracts were washed with water (100 ml × 3), brine (100 ml × 2), dried and freed of solvent to afford a dark highly viscous residue (106 g). A small sample was esterified (CH_2N_2), distilled (b.p. 180–190°/1.5 mm; n_D^{30} 1.5130) and analysed by glc (Table 1): at least twelve components having RRT (with respect to methyl hardwickiate) of 0.06 (9.5%), 0.14 (5%), 0.23 (2.5%), 0.33 (5%), 0.39 (3%), 0.60 (11%), 0.70 (5%), 0.83 (17%), 1.00 (42%), 1.21 (3%), 1.37 (0.3%), 1.60 (1.5%).

Hardwickiic acid (12). The above crude acid mixture (105 g) was rapidly distilled under high vacuum to get a pale yellow syrup (96 g): b.p. 208–215°/6.7×10^{-2} mm, $[\alpha]_D$ −102.3°.

To the distilled product (61.2 g) in dry hexane (150 ml), a soln of cyclohexylamine (19.8 g, 0.2 mole) in dry hexane (100 ml) was slowly introduced with good mixing. The clear soln, after being kept at ~0° (24 hr), deposited a crystalline cyclohexylamine salt, which was rapidly collected by filtration, washed well with cold hexane and at once dried in a vacuum dessicator: m.p. 134–140°, yield 55 g. This was recrystallised from dry ether–hexane (1:1) to furnish colourless needles (35.8 g), m.p. 143–144°, $[\alpha]_D^{27}$ −100.24° (*c*, 8.2%). Elemental analysis proved difficult due to its hygroscopic nature. (Found: C, 76.12, 74.54; H, 10.22, 10.18. $C_{26}H_{41}O_3N$ requires: C, 75.86; H, 10.04%). The amine salt (5.0 g) in ether (100 ml) was shaken with a saturated aq oxalic acid soln (50 ml × 2), washed with water, brine and dried. Revoval of solvent yielded a foam (3.6 g), which was crystallised from MeCN to furnish small button-like crystals, m.p. 106–107°, $[\alpha]_D^{27}$ −114.7° (*c*, 4.95%). (Found: C, 75.58; H, 9.04. $C_{20}H_{28}O_3$ requires: C, 75.91; H, 8.92%).

Kolavenic acid. The total mother liquor from the preparation of the cyclohexylamine salts (from 94 g of the distilled acids) was shaken with a saturated aq soln of oxalic acid (100 ml × 3) to liberate acids. The aq part was discarded and the solvent layer extracted with 10% NaOH aq (100 ml × 3). The aq alkaline extract was acidified (conc HCl) and the acids taken up in ether (100 ml × 4) to finally yield, after the usual work-up, 49 g of an acid mixture, This was esterified with CH_2N_2 and the product distilled: b.p. 190–250°/0.2 nm, n_D^{30} 1.5082–1.5195, yield 50 g. Glc (*vide supra*) revealed this material to consist of essentially methyl hardwickiate (35%) and another ester (RRT 0.83, 37%; methyl kovalenate). Chromatography (Al_2O_3/II, 22 cm × 1.5 cm)

of this material (14.8 g) furnished in the light pet eluates, (100 ml × 14; after rejecting first 100 ml × 5 cuts) 4.75 g of pure (tlc, glc) methyl ester of kolavenic acid: b.p. 179–180°/0.4 mm, n_D^{30} 1.5136, $[\alpha]_D$ − 65.6° (*c*, 4.7%). (Found: C, 79.47; H, 11.01. $C_{21}H_{34}O_2$ requires: C, 79.19; H, 10.76%).

Methyl hardwickiate. Hardwickiic acid (1.5 g) in ether (25 ml) was esterified with an ether soln of CH_2N_2 in the usual way and then worked up to give the methyl ester: viscous liquid (1.45 g), b.p. 150–152°/0.1 mm, n_D^{30} 1.5237, d_4^{29} 1.0815, M_D 93.28 (calcd.:[36] 94.4), $[\alpha]_D^{27}$ − 114.6° (*c*, 3.4%). IR: 1715, 1635, 1564, 1503, 1382, 1252, 1235, 1200, 1165, 1082, 1067, 1028, 880, 822, 790, 762 cm^{-1}. Mass (Varian MAT, CH7, 70 eV): *m/e* 330 (M^+, 48%), 283 (15%), 235 (58%), 194 (58%), 151 (30%), 139 (100%), 96 (78%), 81 (75%), 69 (62%), 55 (33%). (Found: C, 76.38; H, 9.27. $C_{21}H_{30}O_3$ requires: C, 76.32; H, 9.15%). Ehrlich test:[13] orange red (immediate)→ green (5 min)→ olive green (24 hr).

Hardwickiol. The above Me ester (664 mg, 0.002 mole) in ether (30 ml) was reduced with a slurry of LAH (344 mg, 0.004 mole) in dry ether (25 ml), first at − 5° (1 hr), then at 25° (2 hr) and finally at reflux (6 hr) and worked up in the usual manner (first EtOAc, then 5% H_2SO_4 aq) to furnish the desired alcohol (-CH=C-COOMe→-CH-CH_2OH): viscous liquid, b.p. 200–203° (bath)/0.2 mm, n_D^{30} 1.5320, $[\alpha]_D^{27}$ − 47.2° (*c*, 5.6%). IR: 3350, 1565, 1500, 1382, 1165, 1130, 1068, 1030, 1000, 878, 783 cm^{-1}. (Found: 79.43; H, 10.25. $C_{20}H_{36}O_2$ requires: C, 79.42; H, 10.00%). *3,5-Dinitrobenzoate*, white crystals (benzene–hexane), m.p. 118–119°. (Found: C, 64.97; H, 6.73. $C_{27}H_{32}O_7N_2$ requires: C, 65.31; H, 6.50%). *Acetate* (Ac_2O-pyridine, 25°, 48 hr; purified by PLC, solvent 5% EtOAc in C_6H_6); b.p. 175–180° (bath)/0.3 mm, n_D^{30} 1.5170, $[\alpha]_D^{27}$ −54.8° (*c*, 3.3%). IR: 1742, 1382, 1367, 1240, 1163, 1068, 1028, 878, 785 cm^{-1} (Found: C, 76.79; H, 9.97. $C_{22}H_{32}O_3$ requires: C, 76.70; H, 9.36%).

Methyl tetrahydrohardwickiate. Methyl hardwickiate (422.2 mg) was hydrogenated over pre-reduced 5% Rh–C (300 mg) or Rh–Pt[31] (50 mg) in gl AcOH (20 ml). The absorption of H_2 ceased after 13 hr when 69 ml of H_2 at 26°/710 mm (2.04 double bond equivalent) had been consumed. Usual work-up furnished a tlc (solvent 10% EtOAc in C_6H_6)/glc (250°) pure product: b.p. 180–190° (bath)/0.5 mm, n_D^{30} 1.5137, $[\alpha]_D^{27}$ − 89.19° (*c*, 6.07%). IR: 1716, 1635, 1380, 1350, 1275, 1250, 1235, 1198, 1080, 1065, 1032, 940, 910, 783, 760 cm^{-1}. (Found: C, 75.78; H, 10.21. $C_{21}H_{36}O_3$ requires: C, 75.40; H, 10.25%).

Methyl hexahydrohardwickiate. Methyl tetrahydrohardwickiate (238 mg) on further hydrogenation over pre-reduced Adam's Pt catalyst (90 mg) in gl. AcOH (15 ml), absorbed 20 ml of H_2 at 26°/711 mm (1.0 double bond equivalent) during 12 hr, when further hydrogen uptake had ceased. Usual work-up gave a tlc/glc pure product: b.p. 180–190° (bath)/0.4 mm, n_D^{30} 1.5015, $[\alpha]_D^{27}$ − 50.9° (*c*, 2.8%). IR: 1740, 1382, 1365, 1320, 1195, 1173, 1145, 1045 cm^{-1}. (Found: C, 75.04; H, 10.76. $C_{21}H_{36}O_3$ requires: C, 74.95; H, 10.78%).

Curtius-Oesterlin degradation of tetrahydrohardwickiic acid. Tetrahydrohardwickiic acid was obtained as a foam, by hydrogenation of hardwickiic acid exactly as described above for its Me ester. A mixture of tetrahydrohardwickiic acid (0.5 g, 1.56 m mole), freshly activated NaN_3 (0.5 g, 7.68 m mole) and pure dry $CHCl_3$ (15 ml) was stirred, cooled to 0° and treated at the same temp. with H_2SO_4 (d = 1.84 at 15.5°, 2.5 ml) dropwise, after which the reaction mixture was stirred and heated at 50° for 2 hr (cessation of N_2 evalution plus additional half hr). The mixture was next cooled(0°), diluted with water (50 ml) and made strongly alkaline with 20% NaOH aq. The $CHCl_3$ layer was removed, the aq phase extracted with $CHCl_3$ (50 ml × 3) and the combined extracts washed with water, brine and dried. Solvent was flashed off to get 300 mg of a product, which was treated with ether (50 ml) and filtered to remove an insoluble material (80 mg, m.p. 185–195°, not investigated further). The clear ether soln was freed of solvent and the residue distilled to get a viscous liquid (130 mg), b.p. 200–210° (bath)/0.2 mm. This product was converted into its *semicarbazone* (pyridine method), m.p. 232–239°. (Found: C, 68.34; H, 9.64. $C_{28}H_{55}O_2N_3$ requires: C, 68.73; H, 10.09%).

The above semicarbazone (0.5 g), oxalic acid (1.5 g), water (20 ml) and toluene (8 ml) were mixed and refluxed, with stirring, for 5 hr. Usual work-up gave the pure ketone as a viscous liquid (403 mg): b.p. 155–165° (bath)/0.1 mm, n_D^{30} 1.5085, $[\alpha]_D^{29}$ + 17.28° (*c*, 5.38%). IR: 1718, 1387, 1312, 1258, 1240, 1142, 1120, 1067, 1055, 1020, 977, 952, 925, 910 cm^{-1}. PMR: Me-C=(2 H singlets at 0.77, 1.09 ppm), Me-C-H (3 H, d, 0.77 ppm, J = 7 Hz), CH_2OCH_2 (4 H, m, 3.0–3.9 ppm). (Found: C, 78.38; H, 11.27. $C_{19}H_{32}O_2$ requires: C, 78.03; H, 11.03%).

Hexahydrohardwickiol. Methyl hexahydrohardwickiate (187.9 mg) in ether (25 ml) was reduced with a slurry of LAH (100 mg) in ether (40 ml) in the usual manner to furnish, after distillation, a colourless viscous liquid, b.p. 160–170° (bath)/4.33×10^{-3} mm, n_D^{30} 1.5152, $[\alpha]_D^{27}$ − 19.2° (*c*, 1.24%). IR: 3350, 1384, 1035, 997, 905 cm^{-1}. (Found: C, 77.82; H, 12.0. $C_{20}H_{36}O_2$ requires: C, 77.86; H, 11.76%).

Dehydrogenation of hardwickiic acid. A mixture of hardwickiic acid (2.5 g) and 10% Pd–C (1.25 g) was heated at 300–320° in a current of CO_2 till evolution of H_2 had ceased (8 hr). Usual work-up gave a product which was distilled: b.p. 80–102°/0.4 mm, n_D^{30} 1.5350, yield 0.55 g; glc (20% diethylene glycol polysuccinate on Chromosorb W; 180°) showed it to consist of at least 12 components of which four were major having RRT 1 (24%), 3.5 (21%), 5.5 (17%), 8.8 (11%). The four major products were separated by preparative glc; the first two components (RRT 1, 3.5) failed to give any complex with trinitrobenzene (TNB) and hence were not investigated further. The material with RRT 5.5 gave a *TNB complex*, yellow needles (EtOH), m.p. 146–147°. (Found: C, 58.12; H, 4.21. $C_{18}H_{15}O_6N_3$ requires: C, 58.53; H, 4.09%). The complex was decomposed by filtering its benzene soln through Al_2O_3/I to finally furnish *1,2-dimethylnaphthalene*, b.p. 70–80° (bath)/0.4 mm, n_D^{30} 1.6116; *picrate*, m.p. 127–128°. (lit.:[32] TNB complex, m.p. 147–148°; picrate, m.p. 129–130°). UV: λ_{max} nm (ϵ) 227 (95,650), 274 (4875), 282 (5210), 305 (825), 319 (680). PMR: Me (3 H, s, 2.4 ppm; 3 H, s, 2.5 ppm).

The material with RRT 8.8 furnished a *TNB complex*, golden yellow needles (EtOH), M.P. 156–157°, mixed m.p. with an authentic sample of TNB complex of *1,2,5-trimethylnaphthalene* was undepressed. (Found: C, 59.82; H, 4.42. $C_{19}H_{17}O_6N_3$ requires: C, 59.53; H, 4.47%). Pure 1,2,5-trimethylnaphthalene obtained by regeneration from the complex had: b.p. 90–110° (bath)/0.4 mm, m.p. 30–31°, n_D^{30} 1.5975; UV: λ_{max} nm (ϵ) 230 (89,620), 277 (5395), 287 (6060), 307 (1330), 322 (978); PMR: Me (3 H singlets at 2.40, 2.50, 2.60 ppm). (lit.:[32] UV, derivatives).

Ozonolysis of hardwickiic acid. Harwickiic acid (10 g) in dry EtOAc (150 ml) was ozonolysed (0.8 g O_3/hr) at − 10° till O_3 was no longer absorbed (KI–AcOH test; 5.5 hr). Solvent was cautiously removed under suction at ≯45°, the residue mixed with H_2O_2 aq (30%, 40 ml), water (50 ml), and heated at 60 ± 5° for 1 hr and later, on a steam-bath for 3 hr. The mixture was worked up in the usual manner with ether and the product esterified with CH_2N_2 to afford a viscous material (10.2 g), showing six spots on tlc (solvent: 20% EtOAc in C_6H_6) and having RR_f 0.08, 0.12, 0.29, 0.61, 0.78 and 1.00. The total material (30.5 g) from three such experiments was chromatographed on SiO_2gel (44 cm × 7.5 cm):

Fraction 1	C_6H_6	500 ml × 12	0.263 g, rejected
	1% EtOAc in C_6H_6	500 ml × 4	
Fraction 2	1% EtOAc in C_6H_6	500 ml × 8	0.795 g, RR_f 1.00
Fraction 3	2% EtOAc in C_6H_6	500 ml × 2	0.295 g, mixture
Fraction 4	2% EtOAc in C_6H_6	500 ml × 24	10.07 g, RR_f 0.78
Fraction 5	2% EtOAc in C_6H_6	500 ml × 12	3.583 g, RR_f 0.61
Fraction 6	2% EtOAc in C_6H_6	500 ml × 50	6.732 g, RR_f 0.61 (major) and 0.29
Fraction 7	5% EtOAc in C_6H_6	500 ml × 4	4.45 g, mixture,
	10% EtOAc in C_6H_6	500 ml × 20	not investigated.

Ester 13. Fraction 2 on distillation furnished 13 as a viscous liquid (750 mg): b.p. 165–175° (bath)/0.8 mm, n_D^{30} 1.4951, $[\alpha]_D^{28}$ − 6.66° (*c*, 5.85%). IR: 1750, 1720, 1260, 1240, 1200, 1170, 1150, 1085, 1068, 1042, 1028 cm^{-1}. (Found: C, 70.31; H, 9.61. $C_{19}H_{30}O_4$ requires: C, 70.77; H, 9.38%).

Ester 14. Fraction 4 was distilled to afford pure 14 as a colourless viscous liquid (8.9 g): b.p. 183°/0.3 mm, n_D^{30} 1.4802, $[\alpha]_D^{28}$ + 3.9° (*c*, 8.18%). IR: 1754, 1252, 1205, 1188, 1149, 1111, 1074, 1031 cm^{-1}. (Found: C, 63.78: H, 8.93. $C_{19}H_{32}O_5$ requires: C, 64.02; H, 9.05%).

Ester **15**. Fraction 5 on distillation yielded **15** as a colourless viscous liquid (3.0 g): b.p. 190–195 (bath)/0.4 mm, n_D^{30} 1.4848, $[\alpha]_D^{28}$ + 6.4° (*c*, 9.7%). IR: 1750, 1735, 1250, 1200, 1180, 1148, 1108, 1070 cm^{-1}. (Found: C, 67.10; H, 9.41. $C_{19}H_{32}O_5$ requires: C, 67.03; H, 9.47%).

Sodium hypobromite oxidation of keto ester **15**. To a stirred soln of **15** (260 mg) in dioxane (5 ml), a soln of NaOBr (from 0.45 g NaOH in 4 ml H_2O, and 0.25 ml Br_2 was added slowly (0.5 hr) under ice-cooling. The mixture was stirred for another 3 hr 0° and then left aside overnight (14 hr) at room temp. (20–25°). Usual work-up (destroying excess NaOBr with $NaHSO_3$, acidification with HCl aq, extraction with ether) afforded a gum, which was esterified (CH_2N_2) and chromatographed (SiO_2 gel, 21 cm × 1.2 cm; see above chromatography) to get **14** (70 mg; identified by IR, tlc) in the 1% EtOAc in C_6H_6 eluates (20 ml × 10).

Unsaturated keto ester **16**. A mixture of **15** (500 mg), NBS (500 mg), K_2CO_3 (100 mg), benzoyl peroxide (20 mg) and CCl_4 (40 ml) was stirred and refluxed (N_2) for 14 hr, the reaction course being monitored by tlc. After the usual work-up the bromo derivative was obtained as a yellow-greenish gum (0.774 g). Bromination was also carried out with Br_2–Et_2O complex[28] (at 0°, 45 min) with similar results.

The bromo derivative (0.77 g), freshly distilled DMF (15 ml) and $CaCO_3$ (0.5 g) were mixed and refluxed (N_2) for 2 hr. The mixture was cooled, diluted with water and worked up with ether to furnish a reddish viscous residue (0.522 g) showing λ_{max} 227 nm (ε 6199). Column cyromatography (SiO_2 gel/III, 18 cm × 1.6 cm; 0–10% EtOAc in C_6H_6 as eluent) furnished in 5% EtOAc in C_6H_6 cuts (10 ml × 5) 100 mg of pure **16**: b.p. 190–210° (bath)/0.5 nm, n_D^{30} 1.4936, $[\alpha]_D^{31}$ + 10.16° (*c*, 4.9%). IR: 1750, 1695, 1645, 1270, 1185 cm^{-1}. PMR: Me–CH (3 H, d, 0.75 ppm, J = 6.5 Hz), Me–C=(3 H singlets at 0.95, 1.22 ppm), MeCO (3 H, s, 2.23 ppm), COOMe (3 H singlets at 3.63, 3.70 ppm), –C–CH=CH–C=O (2 H, ABq, 6.33 ppm, J_{AB} = 16 Hz, $J_{AB}/\delta_B - \delta_A$ = 0.47). (Found: C, 66.76; H, 8.74. $C_{19}H_{30}O_5$ requires: C, 67.43; H, 8.9%).

Hydrindanone ester **20**. To a suspension of NaH (0.7 g, 11.2 m mole) in dry xylene (40 ml), the triester **14** (1.954 g, 5.48 m mole) and a drop of abs EtOH were added. The mixture was stirred and refluxed under absolutely anhyd. conditions under N_2 for 24 hr. The mixture was chilled and treated with 50% AcOH aq (10 ml), further diluted with water (20 ml) and the xylene layer separated. The aq part was extracted with ether (20 ml × 3) and the extract mixed with xylene layer and the whole washed with water, brine and dried. This soln was treated with some amount of ether–CH_2N_2 to esterify any acid formed (arising from hydrolysis during work-up) and then freed of solvent to get the Dieckmann product as a yellow viscous liquid (~2 g). This product was chromatographed (SiO_2 gel/III, 40 cm × 1.6 cm). After rejecting the material eluted with C_6H_6 (100 ml × 2), pure *β-keto ester* (**17**) was obtained from 1% EtOAc in C_6H_6 eluates (100 ml × 9): colourless thick oil (1.38 g), b.p. 186–196° (bath)/0.7 mm, n_D^{30} 1.4895, $[\alpha]_D^{26}$ – 90.23° (*c*, 5.27%). IR: 1775, 1750, 1265, 1225, 1205, 1190, 1170, 1040, 997 cm^{-1}. (Found: C, 67.10; H, 8.78. $C_{18}H_{28}O_5$ requires: C, 66.64; H, 8.70%). $FeCl_3$ test: deep blue colour.

The *β*-keto ester **17** (1.0819 g), water (1 ml), AcOH (1 ml) and conc HCl aq (2 ml) were mixed and refluxed (N_2) for 4.5 hr. The mixture was worked up with ether, the product esterified (CH_2N_2) and the yellow thick liquid (0.853 g), thus obtained, chromatographed over SiO_2gel/III (26 cm × 1.5 cm). After rejecting the material (0.098 g) elluted with C_6H_6 (50 ml × 3), the required ester **20** was obtained from the 1% EtOAc in C_6H_6 cuts (50 ml × 4), yield 0.658 g, b.p. 172–175° (bath)/0.2 mm, n_D^{30} 1.4923, $[\alpha]_D^{32}$ – 82.36 (*c*, 3.3%). λ_{max} 286 nm (ε 104). IR: 1740, 1260, 1240, 1198, 1172, 1135, 1112, 1052, 1033 cm^{-1}. PMR: Me–CH (3 H, d, 0.82 ppm, J = 6 Hz), Me–C= (3 H singlets at 0.83, 0.97 ppm), COOMe (3 H, s, 3.67 ppm). (Found: C, 72.06; H, 9.64. $C_{16}H_{26}O_3$ requires: C, 72.14; H, 9.84%). 2,4-*Dinitrophenylhydrazone* of **20**, orange yellow lustrous needles, m.p. 173–174° (EtOH).

Side chain degradation of keto ester **20**

Ketal **21**. The required ketal **21** was prepared from **20** (342 mg) and 2 - methyl - 2 - ethyl - 1,3 - dioxolane (10 ml) by exchange reaction under *p*-toluenesulphonic acid (5 mg × 6) catalysis and following the general directions of Dauben *et al.*[33] yield 366 mg, b.p. 186–196° (bath)/1 mm, n_D^{30} 1.4939, $[\alpha]_D^{29}$ + 2.51° (*c*, 2.15%). IR: 1750, 1318, 1270, 1230, 1180, 1158, 1110, 1065, 1025 cm^{-1}. PMR: Me–CH (3 H, d, 0.80, J = 6 Hz), Me–C= (3 H singlets at 0.80, 0.93 ppm), COOMe (3 H, s, 3.6 ppm), O–CH_2–CH_2–O (4 H, bs, 3.80 ppm). (Found: C, 69.40; H, 9.58. $C_{18}H_{30}O_4$ requires: C, 69..64; H, 9.74%).

Diphenylethylene **22**. The above ketal (1.55 g, 0.005 mole) in dry ether (50 ml) was added dropwise to a soln of C_6H_5MgBr (from Mg 646 mg, C_6H_5Br 4.0 g, ether) at 0°. The mixture was next stirred and refluxed for 14 hr and worked up in the usual manner with sat NH_4Cl aq. The product was freed of biphenyl by steam distillation and then chromatographed (SiO_2 gel/III, 21 cm × 1.8 cm) to get in C_6H_6 eluates (80 ml × 5), the required *carbinol* as a foam (1.22 g): λ_{max} 263 (ε 1736), 271 (ε 1808), 279 nm (ε 1520). IR: 3500, 1615, 1395, 1315, 1160, 1060, 1040, 920, 760, 705 cm^{-1}. (Found: C, 80.11; H, 8.83. $C_{29}H_{38}O_3$ requires: C, 80.14; H, 8.81%).

The above alcohol (400 mg) was mixed with 98% HCOOH (1.5 ml) and heated on a steam bath for 7 min, cooled, diluted with water and the product (**22**) isolated by usual work-up with ether–benzene (1:1): viscous liquid (390 mg), λ_{max} 250 nm (ε 9000). The product was purified by chromatography over SiO_2 gel: colourless gum, b.p. 230–235° (bath)/0.1 mm, $[\alpha]_D^{29}$ – 12.74° (*c*, 3.68%); λ_{max} 252 nm (ε 12,875). IR: 1740, 1600, 1380, 1110, 1075, 1046, 1030, 997, 775, 765, 703 cm^{-1}. PMR: Me–CH (3 H, d, 0.72 ppm, J = 6 Hz), Me–C= (3 H singlets at 0.78, 0.90 ppm), C=CH–CH_2 (1 H, t, 6.03 ppm, J = 7.5 Hz), Ar–H (10 H, m, 6.91–7.36 ppm). (Found: C, 86.48; H, 8.78. $C_{27}H_{32}O$ requires: C, 87.05; H, 8.66%).

Keto ester **25**. The above olefine (**22**, 520 mg) was ketalized by exchange with 2 - methyl - 2 - ethyl - 1,3 - dioxalane (20 ml) in the manner described above for **21** to get the ketal (0.570 g) as a gum. This material, without purification, was brominated with NBS (265 mg) in refluxing CCl_4 (4 hr). Usual work-up gave a product (586 mg), which was stirred and heated (~100°) with Li_2CO_3 (45 mg), dioxane (14 ml) and water (5 ml) for 6 hr. The product (463 mg) obtained after ether workup, was chromatographed (SiO_2 gel, 0–3% EtOAc in C_6H_6): 3% EtOAc in C_6H_6 (100 ml × 3) eluted the required alcohol **23** (217 mg, IR: 3380, 1600, 1380, 1300, 1155, 1120, 1060, 1045, 985, 765, 703 cm^{-1}), which was as such subjected to oxidation with RuO_4.

The above alcohol (334 mg, 0.0012 mole) in acetone (50 ml) was treated with a yellow soln of freshly prepared RuO_4[33] in water (from RuO_2[34] 100 mg, $NaIO_4$ 1.1 g, H_2O 4 ml) at room temp. (28°) with stirring. Additional amounts of 25% $NaIO_4$ aq were added as soon as the mixture had turned black, till yellow colour was restored; in this way a total of 2.0 g of $NaIO_4$ was consumed during 15 hr. Isopropanol (15 ml) was added and the reaction mixture stirred for 1 hr to destroy excess RuO_4. The black ppt of RuO_2 was filtered off and the filtrate stripped off solvent under reduced pressure. The residue was taken up in EtOAc and separated into neutral (113 mg, not investigated further) and 5% Na_2CO_3 aq soluble fractions. The aq alkaline soln was acidified (conc HCl aq) and after 2 hr at 0°, the product taken up in EtOAc (50 ml × 3). The EtOAc soln was processed in the usual manner to get 180 mg of an acid, which was esterified (CH_2N_2). This material was passed through a small column of SiO_2 gel: benzene (200 ml) eluted a tlc pure (solvent, 20% EtOAc in C_6H_6) product which was distilled to get pure **25** as a colourless liquid (80 mg), b.p. 150–160° (bath)/1 mm, n_D^{30} 1.4870, $[\alpha]_D^{27}$ – 64.0° (*c*, 3.75%). IR: 1750, 1740, 1272, 1250, 1200, 1175, 1115, 1052 cm^{-1}. PMR: Me–CH (3 H, d, 0.78 ppm, J = 6 Hz), Me–C= (3 H singlets at 0.92, 1.07 ppm), COOMe (3 H, s, 3.65 ppm). (Found: C, 71.00; H, 9.80. $C_{14}H_{22}O_3$ requires: C, 70.55; H, 9.31%). 2,4-*Dinitrophenylhydrazone*, golden yellow needles (EtOH), m.p. 173–174°.

RuO_4 *Oxidation of* **16**. Unsaturated **16** (140 mg) in acetone (20 ml) was oxidized with RuO_4 (RuO_2 40 mg, and a total of 430 mg of $NaIO_4$) at 20–25° during 6 hr, essentially as described above. The crude acid (63 mg), then obtained was esterified (CH_2N_2) and the trimethyl ester (corresponding to **26**) distilled: b.p. 170–175° (bath)/1.0 mm, $[\alpha]_D^{31}$ – 2.47° (*c*, 2.83%). IR: 1737, 1260, 1239, 1200, 1180, 1132, 1100, 1065 cm^{-1}. PMR: Me–CH (3 H, d, 0.73 ppm, J = 6 Hz), Me–C= (3 H, singlets at 1.05,

1.17 ppm), COOMe (3 H, s, 3.60 ppm; 6 H, s, 3.70 ppm). (Found: C, 62.91; H, 8.94. $C_{17}H_{28}O_6$ requires: C, 62.17; H, 8.59).

Hydrindanone ester **28**. The mono-acid diester (220 mg) obtained by RuO_4 oxidation of the unsaturated keto ester **16**, as described above, was exposed to refluxing methanolic KOH (from 100 mg KOH and 10 ml MeOH) for 1 hr, when usual work-up gave the expected diacid mono-ester **26** (196 mg) as a foam. This material (132 mg) was mixed with Ac_2O (2 ml) and heated on a steam-bath for 1 hr. Ac_2O was then distilled off from an oil bath and the cooled residue mixed with fresh Ac_2O (1 ml), refluxed (1 hr) and freed of Ac_2O as before and the residue distilled at 180–200° (bath)/2.0 mm to furnish crude **28** (75 mg). The product was chromatographed on SiO_2 gel/III (2.5 g; 15 cm); 10% EtOAc in C_6H_6 (5 ml × 2) afforded pure (tlc) **28** (17.7 mg): b.p. 160–165° (bath)/1.5 mm, n_D^{30} 1.4839, $[\alpha]_D^{31}$ + 20° (*c*, 0.64%). IR: 1740, 1480, 1250, 1175, 1140, 1112, 1050, 1012 cm^{-1}. PMR: Me–CH (3 H, d, 1.19 ppm, J = 7 Hz), Me–C= (3 H singlets at 0.89, 1.20 ppm), COOMe (3 H, s, 3.65 ppm). (Found: C, 70.85; H, 9.65, $C_{14}H_{22}O_3$ requires: C, 70.55; H, 9.31%).

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REFERENCES

[1]Part VII: *Tetrahedron* **33**, 1759 (1977).
[2]For example see: *The Wealth of India* (Raw Materials), Vol. V, p. 319. Council of Scientific and Industrial Research, New Delhi (1959).
[3]K. S. Iyer and J. J. Sudborough, *J. Indian Inst. Sci.* **2**, 29 (1918).
[4]Sukh Dev and P. C. Guha, *J. Indian Chem. Soc.* **25**, 495 (1948); **26**, 263 (1949).
[5]A. S. Gupta and Sukh Dev, *J. Chromatog.* **12**, 189 (1963).
[6]Available from our previous work: [a]N. P. Damodaran and Sukh Dev, *Tetrahedron* **24**, 4123 (1968); [b]A. S. Gupta and Sukh Dev, *Ibid.* **27** 635 (1971).
[7]A. Aebi, D. H. R. Barton and A. H. Lindsey, *J. Chem. Soc.* 3124 (1953).
[8]Y. Nakamura, T. Shimomura and K. Sawai, *J. Agric. Chem. Soc. Japan* **26**, 493 (1951).
[9]W. Treibs and G. Lossner, *Liebig's Ann.* **634**, 124 (1960).
[10]A. Aebi, D. H. R. Barton and A. S. Lindsey, *J. Chem. Soc.* 3124 (1953); also see *Ref.* (6b.
[11]R. Misra, R. C. Pandey and Sukh Dev, *Tetrahedron Letters* 3751 (1964).
[12]R. Misra, R. C. Pandey and Sukh Dev, *Ibid.* 2681 (1968).
[13]*T. Reichstein, Helv. Chim. Acta* **15**, 1110 (1932).
[14]T. Kubota, *Tetrahedron* **4**, 68 (1958).
[15]E. J. Corey, G. Slomp, Sukh Dev, S. Tobinaga and E. R. Glazier, *J. Am. Chem. Soc.* **80**, 1204 (1958); G. S. Reddy and J. H. Goldstein, *J. Phys. Chem.* **65**, 1539 (1961).
[16]D. E. U. Ekong and J. I. Okogun, *J. Chem. Soc.* (C) 2153 (1969).
[17]V. N. Aiyar and T. R. Seshadri, *Phytochemistry* **11**, 1473 (1973).
[18]W. Cocker, A. L. Moore and A. C. Pratt, *Tetrahedron Letters* 1983 (1965).
[19]G. George, C. Candela, M. Quinet and R. Fellows, *Helv. Chim. Acta* **57**, 1247 (1974).
[20]See e.g.: K. W. Gopinath, T. R. Govindachari, P. C. Parthasarathy and N. Vishwanathan, *Ibid.* **44**, 1040 (1961).
[21]See e.g.: A. T. Nielsen, *J. Org. Chem.* **22**, 1539 (1957).
[22]See e.g.: K. Alder, F. H. Flock, A. Hausweiler and R. Reaber, *Chem. Ber.* **87**, 1752 (1954).
[23]See e.g.: R. M. Carman, *Aust. J. Chem.* **16**, 225 (1963).
[24]See e.g.: J. H. Richard and J. B. Hendrickson, *The Biosynthesis of Steroids, Terpenes and Acetogenins*, p. 240. Banjamin, New York (1964).
I. Fridrichsons and A. McL. Mathieson, *Tetrahedron Letters* No. 26, 18 (1960); W. D. Crow, *Aust. J. Chem.* **15**, 159 (1962).
[26]J. C. W. Chien, *J. Am. Chem. Soc.* **82**, 4762 (1960); J. W. ApSimon, O. E. Edwards and R. Howe, *Can. J. Chem.* **40**, 630 (1962).
[27]See e.g.: R. F. Zürcher, *Helv. Chem. Acta* **46**, 2054 (1963).
[28]N. R. Easton and S. J. Nelson, *J. Am. Chem. Soc.* **75**, 640 (1953).
[29]R. Joly, J. Warnent, G. Nomine and D. Bertin, *Bull. Soc. Chim. Fr.* 366 (1958); R. Joly and J. Warnant, *Ibid.* 367 (1958).
[30]See, e.g.: L. M. Jackman and S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*. p. 165. Pergamon Press, Oxford (1969).
[31]L. Velluz and M. Legrand, *Angew Chem.* **73**, 603 (1961).
[32]T. S. Santhanakrishnan, U. R. Nayak and Sukh Dev, *Tetrahedron* **26**, 641 (1970).
[33]S. Sarel and Y. Yakuka, *J. Org. Chem.* **24**, 2018 (1959); G. Stork, A. Meisels and J. E. Davies, *J. Am. Chem. Soc.* **85**, 3419 (1963); H. Nakata, *Tetrahedron* **19**, 1959 (1963).
[34]A. H. Kapadi and Sukh Dev, *Tetrahedron Letters* 1171 (1964).
[35]See e.g.: A. Windaus and O. Dalmer, *Ber. Dtsch. Chem. Ges.* **52**, 162 (1919).
[36]H. G. Blanc, *Bull. Soc. Chim. Fr.* **3**, 778 (1908).
[37]M. Ferrari, F. Pelizzoni and G. Ferrari, *Phytochemistry* **10**, 3267 (1971).
[38]A. Ohsuka, S. Kusumoto and M. Kotake, *J. Chem. Soc. Japan* 631 (1973).
[39]M. S. Henderson, R. D. H. Murray, R. McCrindle and D. McMaster, *Can. J. Chem.* **51**, 1322 (1973).
[40]J. T. Pinhey, R. F. Simpson and I. L. Batey, *Aust. J. Chem.* **25**, 2621 (1972).
[41]P. R. Jefferies, J. R. Knox and B. Scaf, *Ibid.* **26**, 2199 (1973).
[42]A. B. Anderson, R. McCrindle and E. Nakamura, *J. Chem. Soc. Chem. Comm.* 453 (1974); G. Ferguson, W. C. Marsh, R. McCrindle and E. Nakamura, *Ibid.* 299 (1975).
[43]D. Billet, M. Durgeat, S. Heitz, J. P. Brouard and A. Ahond, *Tetrahedron Letters* 2773 (1976).
[44]S. R. Wilson, L. A. Neubert and J. C. Huffman, *J. Am. Chem. Soc.* **98**, 3669 (1976).
[45]See e.g.: R. McCrindle and K. H. Overton, *Rodd's Chemistry of Carbon Compounds* (Edited by S. Coffey), Vol. IIc, p. 377. Elsevier, Amsterdam (1969).
[46]D. D. Evans and C. W. Shoppee, *J. Chem. Soc.* 543 (1953).
[47]H. Brockmann and H. Schodder, *Ber. Dtsch. Chem. Ges.* **74**, 73 (1941).
[48]See e.g.: Sukh Dev. *J. Indian Chem. Soc.* **30**, 668 (1953).
[49]See, e.g.: Ref. 6.
[50]A. K. Auwers and F. Eisenlohr, *Ber. Dtsch. Chem. Ges.* **43**, 806 (1910).
[51]S. Nishimura, *Bull. Chem. Soc. Japan* **33**, 566 (1960); **34**, 1544 (1961).
[52]J. E. Faraday, *Faraday's Encyclopedia of Hydrocarbon Compounds*, Vols. 10, 12, Chemindex, London (1953, 1955).
[53]H. J. Dauben, B. Löken and H. J. Ringold, *J. Am. Chem. Soc.* **76**, 1359 (1954).
[54]F. S. Martin, *J. Chem. Soc.* 3058 (1952).