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STUDIES ON CONSTITUENTS OF *ISODON JAPONICUS* HARA

THE STRUCTURES AND ABSOLUTE STEREOCHEMISTRY OF ISODONAL, TRICHODONIN AND EPINODOSIN

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Abstract—Seven diterpenoids have been isolated from *Isodon japonicus* Hara. Isodonal, trichodonin and epinodosin are formulated by chemical and spectroscopic evidence as the structures **2**, **4** and **5**. Antimicrobial activity has been tested.

In the preceding paper it was shown the ether extract of the leaves of *Isodon japonicus* Hara (Labiatae) contains many enmein (**1**)[1] type diterpenoids except oridonin (**6**)[2] which has full *ent*-kaurene skeleton. From this plant nine diterpenoids have so far been isolated and their structures characterized by Fujita *et al.*[2-6] We have isolated the seven diterpenes, listed in Table 1, from the same plant. Our results, some of which have been briefly reported previously[7,8] are now described in full. All compounds show correct analytical data for diterpenes or their acetates and all except trichodonin have bitter tastes. Their molecular formulae and some physical properties are shown in Table 1. Antimicrobial activity of five natural compounds **1**, **2**, **3**, **6** and **7** have been checked. The results of this are shown in Table 2. All compounds exhibited a highly specific antibacterial activity against gram-positive bacterium, a strain of *Bacillus subtilis*.

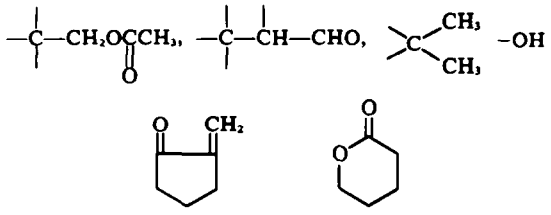
Structure of isodonal.[7] A minor bitter principle isolated from *I. japonicus* Hara, was shown to be a new enmein type diterpene and was designated isodonal (**2**). The molecular formula was established by the mass spectrum and by elemental analysis as $C_{22}H_{28}O_7$. Isodonal exhibits a UV absorption at 230·5 nm (log ϵ 3·75), IR bands at 1710 and 1640 cm^{-1}, and 100 MHz NMR signals at 6·01 and 5·45δ (1H, singlets), typical of a 5-membered ring ketone conjugated with an exocyclic methylene group, as in the D-ring of enmein (**1**). The IR spectrum also shows absorption bands of a free OH group and a 6-membered lactone at 3540 and 1740 cm^{-1}, respectively. The presence of a primary acetoxyl group is evident from IR bands at 1740 and 1240 cm^{-1} and from NMR absorptions at 2·02δ (3H, singlet) and 5·08δ (2H, AB-quartet, J_{AB} = 13 Hz). The NMR spectrum also indicates the presence of a secondary aldehyde at 9·90δ (1H, doublet, J = 3 Hz), whose environment was confirmed by spin decoupling. On irradiation at 4·05δ (1H, doublet, J = 3 Hz), the doublet at 9·90δ collapsed to a singlet. In addition the NMR spectrum exhibits evidence for a gem-dimethyl group, 1·10 and 1·01δ (3H, singlets) and for two methine protons on oxygenated carbons, 4·07δ (1H, doublet of doublets, J_{AX} = 10 and J_{BX} = 12 Hz) and 5·35δ (1H, multiplet).

The above spectroscopic data indicate the presence of the following partial structures in isodonal (**2**); thus characterizing all O atoms.

On catalytic hydrogenation in dioxan over 10% Pd-C, isodonal took up one mole of hydrogen to give dihydroisodonal (**8**), $C_{22}H_{30}O_7$, whose UV spectrum confirms reduction of the exocyclic methylene group. Acetylation of isodonal with acetic anhydride in pyridine yielded acetylisodonal

Table 1

Name	Molecular formula	M.p.	$[\alpha]_D$
Enmein (**1**)[1]	$C_{22}H_{26}O_6$	305–310° (decomp.)	
Isodonal (**2**)[7]	$C_{22}H_{28}O_7$	245–247° (decomp.)	+91·8°
Nodosin (**3**)[3]	$C_{20}H_{26}O_6$	276–280° (decomp.)	−198·0°
Trichodonin (**4**)[8]	$C_{22}H_{28}O_7$	245–247° (decomp.)	+10·0°
Epinodosin (**5**)[8]	$C_{20}H_{26}O_6$	267–271° (decomp.)	−173·7°
Oridonin (**6**)[6]	$C_{20}H_{28}O_6$	250–252° (decomp.)	−45·5°
Enmein-3-acetate (**7**)[4]	$C_{22}H_{28}O_7$	265–271° (decomp.)	−110·1°



(**9**), $C_{24}H_{30}O_8$. The IR spectrum of the latter compound contains no OH band, while in the NMR spectrum the signals of newly produced acetoxyl and methine protons of a $-\overset{|}{C}H-O-COCH_3$ group are observed. The secondary nature of the OH group in isodonal (**2**) is confirmed by its oxidation of **8** to a ketone (see below). The acetoxyl groups in **9** could not be hydrolysed with oxalic acid in contrast to diacetylenmein, in which the acetoxyl group of the hemiacetal is easily hydrolysed under the same conditions. Hydrogenation of acetylisodonal (**9**) over 10% Pd-C gave acetyldihydroisodonal (**10**), $C_{24}H_{32}O_8$, which was obtainable in turn, by acetylation of **8**.

Oxidation of **8** with Jones' reagent afforded, by oxidation of the OH group, an aldehydeketone (**11**), $C_{22}H_{28}O_7$, as the major product and, by oxidation of the aldehyde group, a hydroxycarboxylic acid (**12**), $C_{22}H_{28}O_8$, as the minor product. The latter compound has hydroxyl and carboxylic acid bands at 3400, and 3200, 2800–2500 and 1690 cm^{-1} respectively in the IR spectrum and gave a monomethylester (**13**), $C_{23}H_{30}O_8$, which displays in the NMR spectrum a signal for a carbomethoxyl group at 3·69δ (3H, singlet) but not the one due to the aldehyde group. This monomethylester yielded an acetylmonomethylester (**14**), $C_{25}H_{32}O_9$, which has no IR band due to OH group. The former still shows the signal of an aldehyde group at 9·88δ (1H, doublet, J = 3 Hz), while the OH band which had been observed in isodonal and dihydroisodonal disappeared creating a new band due to a 6-membered ring ketone at 1710 cm^{-1}. The formation of aldehydeketone **11** confirms that the OH group in isodonal is secondary.

The presence of an aldehyde and acetoxyl groups and the absence of a hemiacetal ring in isodonal are large structural differences from enmein. However, the foregoing results included the NMR data in the Table 3 could be satisfied only by an enmein type skeleton for isodonal and the partial structure and

Table 2. Activity of natural compounds on growth of microorganisms

Compounds[a]	Microorganisms[b] I	II	III	IV
None	++[c]	++	++	++
1	+	−[d]	++	++
2	+	−	++	++
3	+	−[e]	++	++
6	+	−[f]	++	++
7	+	−[g]	++	++

[a] Added at 100 ppm.
[b] I, *Escherichia coli*; II, *Bacillus subtilis*; III, *Saccharomyces cerevisiae*; IV, *Penicillium crustosum*.
[c] ++, No effect; +, Partial inhibition; −, Complete inhibition.
[d] Effect at 20 ppm.
[e] Effect at 10 ppm.
[f] Effect at 10 ppm.
[g] Effect at 20 ppm.

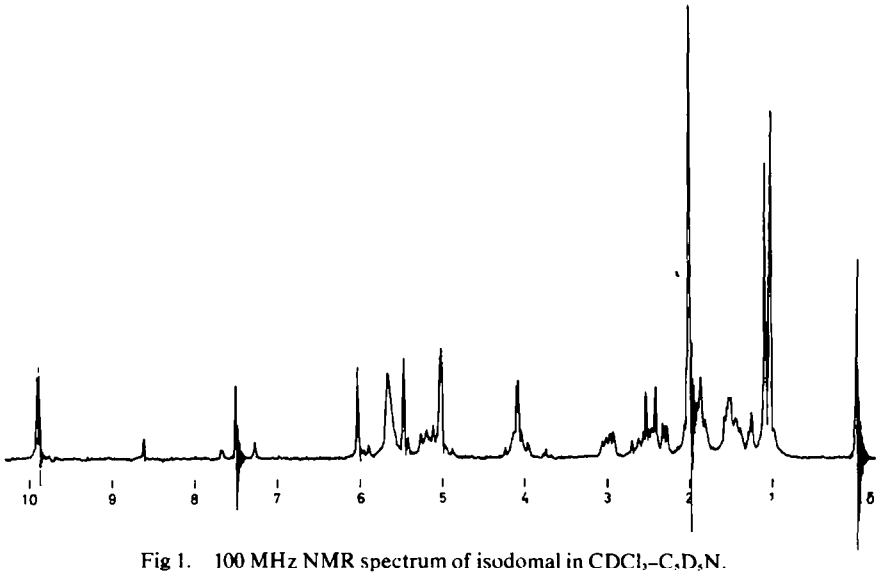
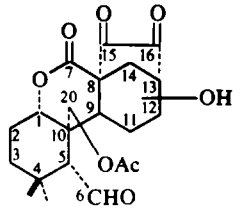


Fig 1. 100 MHz NMR spectrum of isodomal in $CDCl_3$–C_5D_5N.

absolute configuration can therefore be represented by 2_A.



2A

The remaining uncertainty in 2_A is the position and stereochemistry of the secondary OH group. In the NMR spectrum of **11**, the C_1 proton appears at 5·32δ as a doublet of doublets and this means that the C_1 proton in **11** is coupled with two neighboring protons. Furthermore, the CO group at C_3 could be expected to deshield the $C_{4\beta}$ Me group just as bisdehydrodihydroenmein (**15**) which was obtained from enmein.[1] The NMR signal of gem-dimethyl of **11** appears as a sharp singlet at 1·17δ (6H). These observations excluded the possibility of the presence of the CO group on the A-ring, that is, the position of the original OH group at C_2 or C_3. It must therefore be on C-ring. The fact **11** produces a strongly positive Zimmermann test and this suggests the presence of at least one methylene group adjacent to the ketone. Since the position C_{14} is excluded, only two positions, C_{11} and C_{12}, could account for these results. From the biogenetic basis, it is most reasonable to place it at C_{11}.

To prove this assignment isodonal was correlated with nodosin (**3**) by the following manner. Dihydroisodonal (**8**) was hydrolysed with 2N HCl in ethanol to give a hemiacetal (**16**), $C_{20}H_{28}O_6$, which gave a diacetate (**17**), $C_{24}H_{32}O_8$. The formation of the hemiacetal group in **16** was substantiated by its NMR spectrum, which shows a signal at 6·43δ (1H, singlet) due to the C_6 methine proton instead of the aldehyde proton in isodonal. Appearance of the C_6 methine proton as a singlet indicates a β-configuration for the hemiacetal OH as in enmein and nodosin, in which the dihedral angle between the protons at C_5 and C_6 is roughly 90°. The newly produced secondary Me group at C_{15} is presumably α, since the hydrogenation is assumed to occur from the less hindered side. Oxidation of **16** with Jones' reagent afforded the ketodilactone (**18**), $C_{20}H_{24}O_6$, identical in all respects with bisdehydrodihydronodosin which was obtained from dihydronodosin (**19**) by oxidation with Jones' reagent. However, **16** is not identical with dihydronodosin. Since the configuration of the C_{11} hydroxyl in **16** in nodosin was determined to be β by Fujita *et al.*, that of the C_{11} hydroxyl in **16** and in isodonal itself is α (S-configuration). This is supported by the observation that in the NMR spectrum of isodonal, a signal for the proton attached C_{11} appears centered at 4·07δ as doublet of doublets ($W_{1/2}$ = 28 Hz) as compared with centered at 5·02δ as multiplet ($W_{1/2}$ = 14 Hz) for nodosin.

The key step in the correlation of isodonal with nodosin was the selective hydrolysis of the dihydroisodonal **8** to give the hemiacetal **16**. As mentioned above, acid hydrolysis in ethanol was the best condition for this purpose, while in dioxane, hydrolysis gave a vinylether (**20**), $C_{20}H_{26}O_5$, whose structural assignment was derived from a parent ion at *m/e* 346, an IR band at 1640 cm^{-1} and a NMR signal at 6·60δ (1H, singlet).

Treatment of **8** with 0·1% NaOH in methanol gave only a small amount of the desired compound, the main product being an unexpected material (**21**), $C_{21}H_{32}O_7$, which was also obtainable in good yield by the action of LAH in methanol. The structure of this compound was elucidated as **21** from the following data. The IR spectrum of **21** shows the presence of a free OH but no acetoxyl group. Oxidation of **21** with Jones' reagent yielded the ketodilactone (**22**), $C_{21}H_{30}O_7$, which exhibits an IR absorption band for a newly produced five membered lactone at 1780 cm^{-1}. Acetylation of **21** with acetic anhydride in pyridine afforded a diacetate (**23**), $C_{25}H_{36}O_9$, which has no IR band due to the hydroxyl group. In the 100 MHz NMR spectrum of **23** a signal at 3·57δ (3H, singlet) due to a carbomethoxyl group, is observed. This can be rationalised by postulating a cleavage of the D-ring resulting from an attack of methoxide anion on the β-ketolactone group of **8**. The NMR spectrum also indicates the presence of a hemiacetal proton at 5·17δ (1H, doublet, J = 10 Hz) assigned to the C_6 methine proton, whose appearance as a doublet indicates that the dihedral angle between the protons at C_5 and C_6 no longer approximates to 90°. One other significant feature of the NMR spectrum is a signal at 4·50δ (2H, AB-quartet, J_{AB} = 20 Hz) due to the $-\overset{|}{\underset{|}{C}}-CH_2-O-$ group at C_{20} whose lower field doublet appears as a doublet of doublets from long range coupling with the $C_{9\alpha}$ proton (1H, J = 1 Hz) in a relationship very close to the "planar W".

The C-ring in **21** and its acetate **23** should be a strain free chair form and the stereochemistry at C_{11} was confirmed as follows. The C_{11} proton in **23** appears at 4·28δ (1H, doublet of triplets, $J_{ax\text{-}ax}$ = 16 and $J_{ax\text{-}eq}$ = 4·5 Hz) and the large coupling constants support axial positioning of the $C_{9\alpha}$, $C_{11\beta}$ and $C_{12\alpha}$ protons. This is further evidence that the C_{11} proton is β-orientated in isodonal itself.

Structure of trichodonin.[8] Trichodonin was isolated from *I. trichocarpus* Kudo and on the basis of spectroscopic data and biogenetic considerations the structure (**4**) was deduced for it by Fujita *et al.* We have isolated the same compound from *I. japonicus* Hara and have confirmed unambiguously this structural assignment by correlation with isodonal (**2**).

Table 3. NMR spectra for some derivatives of isodonal (δ in ppm)[a]

	Compounds				
	9	**10**	**11**	**14**	**18**
$(CH_3)_2C$	1·18(s)	1·18(s)	1·17(s)	1·00(s) 1·19(s)	1·10(s) 1·29(s)
CH_3CH	—	1·12(d, J = 7)	1·27(d, J = 7)	1·25(d, J = 7)	1·31(d, J = 7)
$COC{=}CH_2$	6·15(s) 5·63(s)	—	—	—	—
CCH_2O	5·10(s)	5·20(s)	4·90(s)	5·15(s)	4·25(d, J = 3)
CH—CHO	3·04(d, J = 3)	2·85(d, J = 3)	3·35(d, J = 3)	—	—
CH—CHO	9·88(d, J = 3)	9·88(d, J = 3)	9·87(d, J = 3)	—	—
$OCOCH_3$	2·13(s) 2·00(s)	2·13(s) 2·00(s)	2·02(s)	2·05(s) 2·26(s)	—
CHOAc	4·88(dd, J_{AX} = 7, J_{BX} = 15)	4·88(dd, J_{AX} = 7, J_{BX} = 15)	—	4·98(m)	—
$COOCH_3$	—	—	—	3·69(s)	—

[a]Determined in $CDCl_3$ at 60 MHz. Coupling constants are expressed in Hz. s: singlet, d: doublet, dd: doublet of doublets, m: multiplet.

The molecular formula, $C_{22}H_{28}O_7$ established by the mass spectrum and elemental analysis, is the same as that of isodonal. Their close structural relationship is evident from the similarity of their UV, IR and NMR spectra. Thus, seven of the oxygen atoms in trichodonin were characterised as belonging to the same functional groups as those of isodonal.

On catalytic hydrogenation over 10% Pd-C, followed by oxidation with Jones' reagent both isodonal and trichodonin yielded the same aldehydeketone **11**. The two compounds are thus epimeric at C_{11}. Reduction of **11** with $NaBH_4$ in methanol afforded exclusively dihydroisodonal **8**. Assuming hydride ion approach from the less hindered side, this would indicate that the C_{11} OH is α orientated in isodonal and β in trichodonin.

It is interesting to note that isodonal and trichodonin are epimers at C_{11} occuring in the same species and that isodonal has strong bitter taste but trichodonin has no taste.[9]

Structure of epinodosin. I. japonicus Hara contains at least one further pair of C_{11} epimer, in addition to nodosin (**3**), we have isolated epinodosin (**5**), $C_{20}H_{26}O_6$. O-Ethylepinodosin (**24**), $C_{22}H_{30}O_6$ was also obtained, which was probably formed during extraction and purification. Acetylation of **24** with acetic anhydride in pyridine gave O-ethylepinodosin acetate (**25**), $C_{24}H_{32}O_7$. The presence of OEt group in **25** is evident from NMR absorption at 1·12δ (3H, triplet, J = 7 Hz) and 3·50δ (2H, quartet, J = 7 Hz). O-Ethylepinodosin was hydrogenated over 10% Pd-C to dihydro-O-ethylepinodosin (**26**), $C_{22}H_{32}O_6$ and hydrolysed with oxalic acid giving dihydroepinodosin (**16**), $C_{20}H_{28}O_6$. This was identical with the hemiacetal compound **16** produced by acid hydrolysis in ethanol, demonstrating that epinodosin **5** is the C_{11} epimer of nodosin **3** and that it has the $C_{11\alpha}$ OH group (R-configuration).

EXPERIMENTAL

NMR spectra were determined at either 60 MHz using a JEOL 60HL or at 100 MHz using a JEOL PS-100 spectrometer in $CDCl_3$ solns, unless otherwise stated. Mass spectra were obtained on a Hitachi RMU-6. IR spectra were recorded on Nujol mull with a Japan Spectroscopic IR-S spectrometer. UV data were measured in EtOH soln with a Hitachi EPS-2 spectrometer. A Rex Optical Works model NEP-2 was used to measure the $[\alpha]_D$ using pyridine as solvent. Column chromatography was performed on either Mallinckrodt silicic acid or neutral alumina. The preferred adsorbent was deactivated alumina (Activity III) prepared by the addition of water as directed by the manufacturers. M.ps were taken in glass capillaries and uncorrected.

Isolation. The dried leaves (6·7 kg) of *Isodon japonicus* Hara growing on the Botanical Garden of Osaka City University were extracted with ether (30 l). The crude extract was treated with activated charcoal, concentrated and left to cool overnight. Then a solid (105 g) was filtrated off. The filtrate was chromatographed on alumina (2 kg). Elution with ether gave isodonal, trichodonin, O-ethylepinodosin, enmein-3-acetate, nodosin, epinodosin, enmein and oridonin in this order.

Isodonal **2** (5·09 g) formed fine needles from EtOH, m.p. 245–247° (dec), $[\alpha]_D^{20}$ +91·8° (c = 1·0, pyridine), UV(EtOH) 230·5 nm (log ϵ 3·75), IR(Nujol) 3540, 1740, 1710, 1640, 1240, 1080, 970 and 820 cm^{-1}, NMR (Fig 1), Mass m/e 404(M^+), 386(M-18), 376(M-28), 362, 344, 311, 298, 273, 245, 227, 217, 199, 150, 149. (Found: C, 65·40; H, 7·02. $C_{22}H_{28}O_7$ requires: C, 65·33; H, 6·98%).

Trichodonin **4** (52 mg) formed plates from EtOH, m.p. 245–247° (dec), $[\alpha]_D^{20}$ + 10·0° (c = 1·0, pyridine), UV(EtOH) 233 nm (log ϵ 3·78), IR(Nujol) 3480, 1750, 1705, 1640, 1250, 1080, 970 and 830 cm^{-1}. (Found: C, 65·37; H, 7·17. $C_{22}H_{28}O_7$ requires: C, 65·33; H, 6·98%). It was identified by IR comparison with an authentic sample.

Enmein-3-acetate **7** (140 mg) formed needles from EtOH, m.p. 265–271° (dec), $[\alpha]_D^{20}$ − 110·1° (c = 1·0, pyridine), 233 nm 23h nm (log ϵ 3·82), IR(Nujol) 3450, 1745, 1705, 1640 and 1250 cm^{-1}. (Found: C, 65·52; H, 7·29. $C_{22}H_{28}O_7$ requires: C, 65·33; H, 6·98%). It was identified by IR comparison with an authentic sample.

Nodosin **3** (4·85 g) formed plates from EtOH, m.p. 276–280° (dec), $[\alpha]_D^{20}$ − 198·0° (c = 1·0, pyridine), UV(EtOH) 233 nm (log ϵ 3·77), IR(Nujol) 3520, 3440, 1740, 1700, 1640, 1045, 1020 and 885 cm^{-1}. (Found: C, 66·29; H, 7·27. $C_{20}H_{26}O_6$ requires: C, 66·28; H, 7·23%). It was identified by NMR and IR comparison with an authentic sample.

Epinodosin **5** (26 mg) formed needles from EtOH, m.p. 267–271° (dec), $[\alpha]_D^{19}$ − 173·7° (c = 1·0, pyridine), UV(EtOH) 230·5 nm (log ϵ 3·80), IR(Nujol) 3350, 1745, 1710, 1640, 1050 and 880 cm $^{-1}$. (Found: C, 66·38; H, 7·34. $C_{20}H_{26}O_6$ requires: C, 66·28; H, 7·23%).

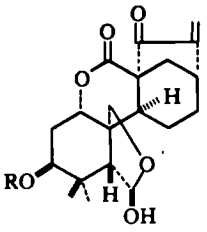
O-Ethylepinodosin **24** (34 mg) formed plates from EtOH, m.p. 204–208° (dec), $[\alpha]_D^{20}$ − 32·6° (c = 1·0, pyridine), UV(EtOH) 230·5 nm (log ϵ 3·88), IR(Nujol) 3420, 1750, 1710 and 1645 cm^{-1}. (Found: C, 67·77; H, 7·77. $C_{22}H_{30}O_6$ requires: C, 67·67; 7·74%).

Enmein **1** (18·5 g) formed plates from aqueous EtOH, m.p. 305–310° (dec). (Found: C, 66·25; H, 7·22. $C_{20}H_{26}O_6$ requires: C, 66·28; H, 7·23%). It was identified by NMR and IR comparison with an authentic sample.

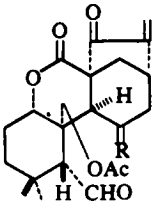
Oridonin **6** (112 mg) formed plates from EtOH, m.p. 250–252° (dec), $[\alpha]_D^{20}$ − 45·5° (c = 1·0, pyridine), UV(EtOH) 238 nm (log ϵ 3·90), IR(Nujol) 3450–3200, 1705, 1650, 1100, 1080 and 1070 cm^{-1}. (Found: C, 65·86; H, 7·80. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%). It was identified by IR comparison with an authentic sample.

Hydrogenation of isodonal (**2**). Isodonal **2** (500 mg) in dioxane (50 ml) was hydrogenated over 10% Pd-C (50 mg) for 24 h. The catalyst was removed by filtration. Then the filtrate was evaporated and crystallized from ethanol to give dihydroisodonal **8** (475 mg), as needles, m.p. 252–253° (dec), $[\alpha]_D^{20}$ + 103° (c = 1·0, pyridine), UV(EtOH)-, IR(Nujol) 3500, 2740, 1740, 1705 and 1240 cm^{-1}. (Found: C, 65·38; H, 7·68. $C_{22}H_{30}O_7$ requires: C, 65·30; H, 7·54%).

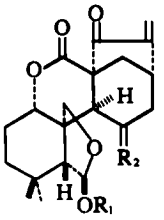
Acetylation of isodonal (**2**). A soln of isodonal **2** (360 mg) in Ac_2O (3 ml) and pyridine (1 ml) was allowed to stand at room temp for 24 h and worked up in the usual way to give a white residue. which was recrystallized from EtOH to give **9** (354 mg), as fine needles, m.p. 228–229° (dec), $[\alpha]_D^{20}$ + 117·7° (c = 1·0, pyridine), IR(Nujol) 1740, 1720, 1705, 1640 and 1240 cm^{-1}, NMR (see Table 3), (Found: C, 64·67; H, 6·84. $C_{24}H_{30}O_8$ requires: C, 64·56; H, 6·77%).



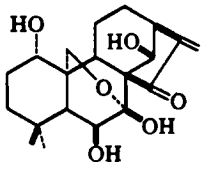
1: R = H
7: R = Ac



2: R = 
4: R = 
9: R = 



3: R_1 = H, R_2 = 
5: R_1 = H, R_2 = 
24: R_1 = CH_2CH_3, R_2 = 
25: R_1 = CH_2CH_3, R_2 = 



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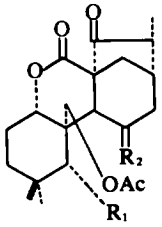
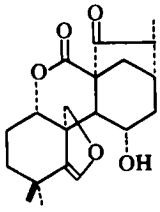


8: R_1 = CHO, R_2 = 
10: R_1 = CHO, R_2 = 
11: R_1 = CHO, R_2 = O
12: R_1 = COOH, R_2 = 
13: R_1 = $COOCH_3$, R_2 = 
14: R_1 = $COOCH_3$, R_2 = 



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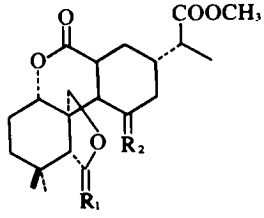
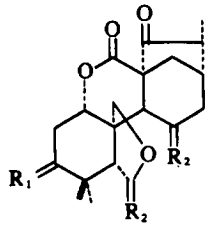


21: R_1 =  , R_2 = 
22: R_1 = R_2 = O
23: R_1 =  , R_2 = 



15: R_1 = R_2 = O, R_3 = H_2
16: R_1 = H_2, R_2 =  , R_3 = 
17: R_1 = H_2, R_2 =  , R_3 = 
18: R_1 = H_2, R_2 = R_3 = O
19: R_1 = H_2, R_2 =  , R_3 = 
26: R_1 = H_2, R_2 =  , R_3 = 

Hydrogenation of acetylisodonal **9**. Acetylisodonal **9** (120 mg) was hydrogenated as described above to give **10** (115 mg), m.p. 200–202° (dec), IR(Nujol) 1740, 1720, 1705 and 1240 cm^{-1}, NMR (Table 3). (Found: C, 64·15; H, 7·35. $C_{24}H_{32}O_8$ requires: C, 64·27; H, 7·19%).

Acetylation of dihydroisodonal **8**. Dihydroisodonal **8** (102 mg) was treated as described above to give **10** (100 mg), identical with an authentic sample.

Oxidation of dihydroisodonal **8**. To a soln of **8** (303 mg) in acetone (80 ml) was added Jones' reagent dropwise with stirring and ice cooling. The reaction was complete within 10 min, the mixture was stirred for further 30 min, then worked up by dilution with water (80 ml) and concentrated *in vacuo* to remove acetone. The white ppt was filtrated and washed with water. The following products were isolated by column chromatography on silicic acid:

(1) Elution with chloroform gave high R_f material (188 mg) which was recrystallized from EtOH to give **11** (156 mg), as fine needles, m.p. 205–206° (dec), IR(Nujol) 2750, 1740, 1710 and 1240 cm^{-1}, NMR (Table 3), strongly positive Zimmermann test. (Found: C, 65·50; H, 7·10. $C_{22}H_{28}O_7$ requires: C, 65·33; H, 6·98%).

(2) Further elution with 30% MeOH in chloroform yielded low R_f material (56 mg) which was recrystallised from EtOH to give **12** (52 mg), as powder, m.p. 263–267° (dec), IR(Nujol) 3400, 3200, 2800–2500, 1740, 1710, 1690 and 1245 cm^{-1}. (Found: C, 62·93; H, 6·85. $C_{22}H_{30}O_8$ requires: C, 62·54; H, 7·16%).

Treatment of dihydroisodonal **8** *with oxalic acid.* Dihydroisodonal **8** (200 mg) was treated with oxalic acid (70 mg) in dioxane (2 ml) and water (3 ml), as described before.[1] The product showed the same m.p. and IR spectrum of **8**.

Methylation of **12**. A soln of **12** (72 mg) in ether (50 ml) was treated with ethereal diazomethane in the usual manner and recrystallized from EtOH to give **13** (70 mg), as needles, m.p. 240–243° (decomp.), IR(Nujol) 3400, 1740, 1705 and 1240 cm^{-1}. (Found: C, 63·73; H, 6·87. $C_{23}H_{30}O_8$ requires: C, 63·58; H, 6·90%).

Acetylation of **13**. A soln of **13** (68 mg) in Ac_2O (1 ml) and pyridine (0·5 ml) was treated as usual manner and recrystallized from EtOH to give **14** (67 mg), as needles, m.p. 240–243° (dec), IR(Nujol) 1740, 1720, 1705 and 1240 cm^{-1}, NMR (Table 3). (Found: C, 63·11; H, 6·83. $C_{25}H_{32}O_9$ requires: C, 63·01; H, 6·77%).

Hydrolysis of dihydroisodonal **8**.

(1) To a soln of dihydroisodonal (500 mg) in EtOH (50 ml) was added 2N HCl (30 ml) and refluxed for 5 h. The mixture was concentrated *in vacuo* and extracted with chloroform (150 ml). The extract was washed with water, dried over Na_2SO_4, evaporated and recrystallized from EtOH to give **16** (32 mg), as plates, m.p. 225–227° (dec), $[\alpha]_D^{20} - 140·8°$ ($c = 1·0$, pyridine), IR(Nujol) 3380, 3300, 1745 and 1705 cm^{-1}, NMR (Table 3). (Found: C, 65·90; H, 7·76. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%).

(2) A soln of **8** (202 mg) in dioxane (30 ml) and 2N HCl (30 ml) was treated as described above to give **20** (102 mg), as a needles, m.p. 238–239°, IR(Nujol) 3500, 1750, 1710 and 1640 cm^{-1}, NMR($CDCl_3$) 1·10 and 1·24 (3H, singlets), 1·20 (2H, doublet J = 7), 3·98 and 3·78δ (2H, AB-quartet, J_{AB} = 12 Hz), Mass *m/e* 346(M^+), 331, 316, 301 and 161. (Found: C, 69·93; H, 7·71. $C_{20}H_{26}O_5$ requires: C, 69·34; H, 7·57%).

Acetylation of **16**. A soln of **16** (108 mg) in Ac_2O (2 ml) and pyridine (1 ml) was treated as described above to give **17** (98 mg), as a needles, m.p. 233–238° (dec), IR(Nujol) 1750, 1715 and 1240 cm^{-1}. (Found: C, 64·09; H, 7·39. $C_{24}H_{32}O_8$ requires: C, 64·27; H, 7·19%).

Oxidation of **16**. A solution of **16** (100 mg) in acetone (30 ml) was treated as described above to give **18** (72 mg), as plate, m.p. 198–200°, UV(EtOH)-, IR(Nujol) 1780, 1755 and 1710 cm^{-1}, NMR (see Table 3). (Found: C, 66·82; H, 6·74. $C_{20}H_{24}O_6$ requires: C, 66·65; H, 6·71%). It was identified by mixed m.p., IR and NMR comparison with the bisdehydrodihydronodosin.

Hydrogenation of nodosin **3**. Nodosin **3** (500 mg) in dioxane (30 ml) was hydrogenated as described above to give **19** (386 mg), m.p. 245–248° (dec), IR(Nujol) 3600, 3480, 1750 and 1710 cm^{-1}. (Found: C, 66·18; H, 7·88. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%).

Oxidation of dihydronodosin **19**. A soln of **19** (102 mg) in acetone (30 ml) was treated as described above to give **18** (88 mg), as a needles, m.p. 198°, IR(Nujol) 1780, 1755 and 1710 cm^{-1}, strongly positive Zimmermann test.

Alkali treatment of dihydroisodonal **8**. To a soln of **8** (1·234 g) in MeOH was added 0·1 N KOH (50 ml) dropwise with stirring and ice cooling and allowed to stand overnight in an ice box. The mixture was then acidified with 2 N HCl (3 ml) and concentrated *in vacuo*. The white ppt was filtrated and washed with water. The following products were isolated by column chromatography on silicic acid:

(1) Elution with chloroform gave high R_f material (576 mg) which was recrystallised from acetone to give the pure **21** (386 mg), as fine needles, m.p. 211–212·5°, IR(Nujol) 3350, 3200, 1735 and 1160 cm^{-1}, NMR(C_5D_5N) 4·82(2H, singlet, C—C$\underline{H}_2$OH), 4·28(2H, doublet, J = 7, CH—C$\underline{H}_2$OH), 5·65(3H, singlet, COOC$\underline{H}_3$) and 3·3δ (3H, 3-O$\underline{H}$). (Found: C, 63·59; H, 8·24. $C_{21}H_{34}O_7$ requires: C, 63·29; H, 8·60%).

(2) Further elution with 2% MeOH in chloroform yielded low R_f material (12 mg) which was recrystallized from EtOH to give **16** (8 mg), m.p. 224–226° (decomp.). It was identified by IR comparison with an authentic sample.

(3) Finally elution with 15% MeOH in chloroform afforded the hydroxycarboxylic acid (148 mg), m.p. 277–280° (decomp.), IR(Nujol) 3380, 3250, 2800–2500, 1740 and 1690 cm^{-1}. (Found: C, 62·49; H, 8·32. $C_{20}H_{32}O_7$ requires: C, 62·48; H, 8·39%). Treatment of this with diazomethane gave **21**.

Oxidation of **21**. A soln of **21** (103 mg) in acetone (15 ml) was treated as described above to give **22** (53 mg), as needles, m.p. 200–203° (dec), IR(Nujol) 1780, 1760 and 1710 cm^{-1}. (Found: C, 64·33; H, 7·21. $C_{21}H_{28}O_7$ requires: C, 64·27; H, 7·19%).

Acetylation of **21**. A soln of **21** (80 mg) in Ac_2O (3 ml) and pyridine (1 ml) was treated as described above to give **23** (81 mg), as plates, m.p. 228–232° (dec), IR(Nujol) 3500, 1730, 1705 and 1230 cm^{-1}, NMR($CDCl_3$, 100 MHz) 1·10 and 1·05 (3H, singlets), 1·15 (3H, doublet, J = 7, CH—C$\underline{H}_3$), 2·12 (6H, singlet, COOC$\underline{H}_3$), 4·50 (2H, AB-quartet, J_{AB} = 20, C—C$\underline{H}_2$O—), 5·17 (1H, doublet, J = 10, —CO—C$\underline{H}$—O), 3·57 (3H, singlet, COOC$\underline{H}_3$), 4·82 (1H, doublet of triplets, J_{AX} = 16 and J_{BX} = 4·5 Hz, —C$\underline{H}$—OAc) and 3·7δ (1H, O$\underline{H}$). (Found: C, 62·54; H, 7·64. $C_{25}H_{38}O_9$ requires: C, 62·22; H, 7·94%).

Treatment of dihydroisodonal **8** *with* LAH *in methanol.* To a soln of dihydroisodonal (528 mg) in MeOH (50 ml) was added LAH (330 mg) in small portions with stirring and ice cooling and the mixture was allowed to stand for 4 h. Then 2N HCl (10 ml) and water (20 ml) was added in this order and extracted with chloroform

(150 ml). The extract was washed with water, dried over Na_2SO_4 and crystallized from acetone to give **21** (146 mg), as needles, m.p. 211–212·5°. It was identified by IR comparison with the authentic sample.

Hydrogenation of trichodonin **4**. Trichodonin **4** (120 mg) in dioxane (10 ml) was hydrogenated as described above to give the dihydrotrichodonin (111 mg), as prisms, m.p. 246–248° (dec), IR(Nujol) 3480, 1755, 1705 and 1270 cm^{-1}. (Found: C, 65·52; H, 7·80. $C_{22}H_{30}O_7$ requires: C, 65·30; H, 7·54%).

Oxidation of dihydrotricyodonin. A soln of dihydroisodonal (100 mg) in acetone (10 ml) was treated as described above to give **11** (82 mg), as fine needles, m.p. 203–206° (dec). (Found: C, 65·71; H, 7·22. $C_{22}H_{28}O_7$ requires: C, 65·33; H, 6·98%). It was identified by mixed m.p. and IR comparison with the authentic sample.

Treatment of **11** *with* $NaBH_4$. To a soln of **11** (315 mg) in MeOH (30 ml) was added $NaBH_4$ in small portions with ice cooling and the mixture was allowed to stand at room temp overnight. After removal of the solvent *in vacuo*, the residue was washed with water, dried and chromatographed on silicic acid to isolate the following products:

(1) Elution with chloroform gave high R_f material (11 mg) which was recrystallized from EtOH to give **8**. It was identified by IR comparison with the authentic sample.

(2) Further elution with 5% MeOH in chloroform yielded low R_f material (234 mg) which was recrystallized from EtOH–EtOAc to give the triol (186 mg), as rods, m.p. 241–246° (dec), IR(Nujol) 3350, 1740, 1705 and 1240 cm^{-1}. (Found: C, 64·40; H, 8·40. $C_{22}H_{34}O_7$ requires: C, 64·37; H, 8·35%). It afforded a tetraacetate, m.p. 210–213° (dec), IR(Nujol) 1740, 1710, 1220 and 1240 cm^{-1}, NMR($CDCl_3$) 2·30, 2·19, 2·17 and 2·12 (3H, singlets, $OCOCH_3$), 4·85 (2H, singlet, $C—CH_2OAc$), and 5·35δ (2H, doublet, J = 12 Hz, $CH—CH_2OAc$).

Acetylation of O-ethylepinodosin **24**. A soln of **24** (80 mg) Ac_2O (1 ml) and pyridine (1 ml) was treated as described above to give **25** (72 mg), as needles, m.p. 232–234° (dec), IR(Nujol) 1740, 1710, 1640 and 1240 cm^{-1}, NMR($CDCl_3$) 1·12 (3H, triplet, J = 7, $CH_2—CH_3$), 3·50 (2H, quartet, J = 7, $O—CH_2CH_3$), 2·33 (3H, singlet, $OCOCH_3$), 5·98 and 5·40 (2H, singlets, $CO—C=CH_2$), 4·57 (2H, AB-quartet, J = 7 Hz, $C—CH_2—O$) and 6·35δ (1H, singlet, O—CH—O). (Found: C, 66·68; H, 7·56. $C_{24}H_{32}O_7$ requires: C, 66·65; H, 7·46%).

Hydrogenation of **24**. O-ethylepinodosin **24** (266 mg) in dioxane (50 ml) was hydrogenated as described above to give **26** (243 mg), as needles, m.p. 215–217° (dec), IR(Nujol) 3400, 1740 and 1710 cm^{-1}. (Found: C, 67·50; H, 8·28. $C_{22}H_{32}O_6$ requires: C, 67·32; H, 8·22%).

Hydrolysis of **26** *with oxalic acid.* Dihydro-O-epinodosin **26** (153 mg) was treated with oxalic acid (60 mg) in dioxane (2 ml) and water (2 ml), as described before,[1] to give **16** (84 mg), as needles, m.p. 228–232° (decomp.), IR(Nujol) 3380, 3300, 1745 and 1705 cm^{-1}. (Found: C, 66·17; H, 7·78. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%). It was identified by mixed m.p. and IR comparison with the authentic sample.

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