NORDITERPENE DILACTONES FROM PODOCARPUS SPECIES

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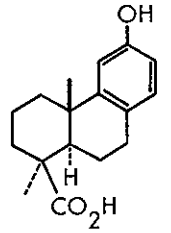
Chemistry of biologically active norditerpene dilactones isolated from Podocarpus species are discussed with emphasis on the relationship between their structures and spectroscopic and other physical properties as well as their biological activities. Also briefly discussed are tetranorditerpene dilactones isolated from a mould.

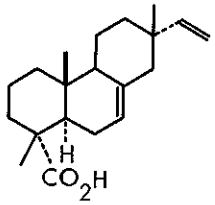
Introduction

The genus Podocarpus (Podocarpaceae) includes 80 species and is distributed in tropical and subtropical area of eastern Asia and southern hemisphere. Some of the species have been the subject of rather extensive chemical investigation in Australia, Japan and New Zealand because of their usefulness as the building material. As the result, various types of compounds, such as lignans, terpenoids and steroids, have been isolated and their structures determined. Diterpenoids isolated are hydrocarbons, phenols, or carboxylic acid which belong to the carbon skeletons of podocarpane, isopimarane, friedoisopimarane, abietane, totarane and 13α- and 13β-kaurane. Some

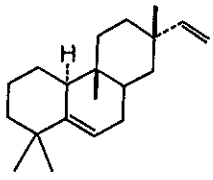
representative examples follow.



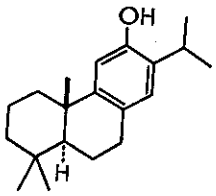
podocarpic acid
(podocarpane)



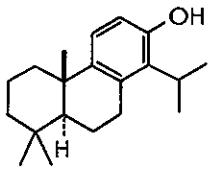
isopimaric acid
(isopimarane)



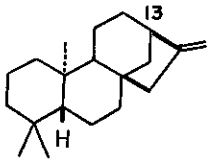
rimuene
(friedoisopimarane)



ferruginol
(abietane)

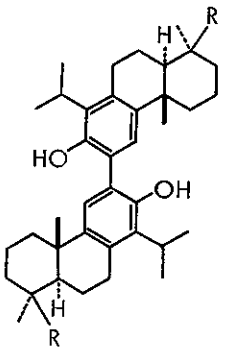


totarol
(totarane)



(+)-kaurene
(kaurane)

Many of these compounds have been isolated only or at least mainly from species in Podocarpaceae and may have taxonomical significance. However, more characteristic constituents in this genus are the C_{40} compounds of dimeric totarane skeleton, although only two compounds, podototarin[1] and macrophyllic acid[2], are presently known.

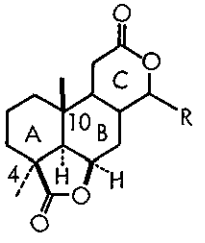


podototarin: R=CH_3
macrophyllic acid: R=COOH

Another feature of taxonomical interest in this genus was uncovered by recent studies: The presence of hardly-soluble norditerpene dilactones, structures of which are closely related each other. By the end of 1974, 25 such compounds have been isolated. In addition to the chemical interest, this group of compounds became rather popular because many of these compounds exhibit important biological activities. The object of the present review is to summerize our knowledge on the chemistry of these lactones, especially spectroscopic properties, in order to help the future investigations in the field.

General Structural Features

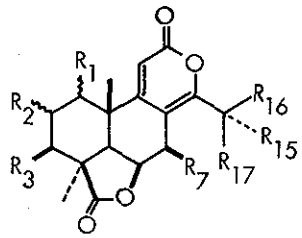
All of the 25 known lactones have the basic ring system shown below of the degraded totarol.



Names of the lactones, their melting points, structures and plant sources are listed in Tables I, II and III.

The structural features common to the whole group is that they have 1) the degraded ring C of totarane carbon skeleton to unsaturated δ-lactone, 2) a γ-lactone between C_{19} carbon and the β-oriented hydroxyl group at C_6, 3) two tertiary methyl groups, α-methyl at C_4 and β-methyl at C_{10}, and 4) extensively oxidized carbocyclic rings A and B with hydroxyl, epoxy and/or olefinic groups. So that all of these dilactones have 19 carbons at largest and up to 9 oxygen atoms in the molecule.

Table 1. Structure of the Lactones in Subgroup A and their Sources

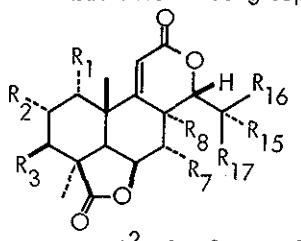


compound No.	names	m.p.*1	sources*2	R 1	2	3	7	15	16	17
1	sellowin C[3]	310° d.	sel			OH	OH	H	Me	Me
2	nagilactone A[4]	305° s.	nag, mac	βOH			OH	H	Me	Me
3	nagilactone C[4]	290° d.	nag, nub sel, niv, hal, mac	-O-		OH	OH	H	Me	Me
4	nagilactone D[4]	265-6° d.	nag	-O-		OH		H	H	Me
5	hallactone A[5]	266-8° d.	hal	-O-		OH		H	Me	Me
6	nagilactone B[4]	258-61° d.	nag	βOH	βOH		OH	H	Me	Me
7	inumaki-lactone E[6]	220-5°	mac	βOH			OH	H, Me		CH_2OH

*1 d: with decomposition, s: with sublimation

*2 sel = P. sellowii Klotzsch, nag = P. nagi Zoll. et Moritzi, mac = P. macrophyllus D. Don, hal = P. hallii Kirk, nub = P. nubigena Lindley, niv = P. nivalis Hook

Table II. Structure of the Lactones in Subgroup B and their Sources

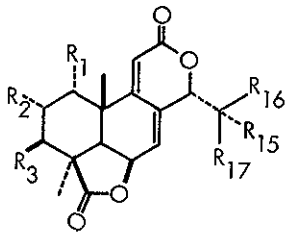


compound No.	names	m.p.*1	sources*2	R 1	2	3	7	8	14	15	16	17
8	compound 8[7]		sel, lam	H	H	H		-O-	~H	H	Me	Me
9	nagilactone E[8]	295°	nag	H	H	OH		-O-	βH	H	Me	Me
10	podolide[9]	296-8°	gra	H	Δ			-O-	βH	H	Me	Me
11	inumaki-lactone A[10]	251-3°d.	mac	-O-		OH		-O-	βH	H	Me	OH
12	sellowin B[3]	316-7°d.	sel, hal	-O-				-O-	βH	CH_2=C		H
13	inumaki-lactone B[11]	295°d.	mac, ner	-O-		OH		-O-	βH	CH_2=C		H
14	sellowin A[3]	298°d.	sel, hal	-O-		H		-O-	βH	OH, CH_3		CH_2OH
15	podolactone[12]	291-3°d.	ner	-O-		H		-O-	βH	OH, CH_3		CH_2OH
16	podolactone B[12]	272-5°d.	ner	-O-		OH		-O-	βH	OH, CH_3		CH_2OH
17	the lactone[13]	259-60°	sal	-O-		H	H	H	~H	H, CH_3		CO_2Me
18	podolactone C[14]	288-90°d.	ner	-O-		H		-O-	βH	OH, CH_3		CH_2SOMe
19	podolactone D[14]	261-6°d.	ner	Δ		H		-O-	βH	OH, CH_3		CH_2SOMe
20	hallactone B[5]	325-30°d.	hal, sel	-O-		H		-O-	βH	OH, CH_3		CH_2SO_2Me

*1 d: with decomposition

*2 sel=P. sellowii Klotzsch, lam=P. lambertii Klotzsch, nag=P. nagi Zoll. et Moritzi, gra=P. gracilior, mac=P. macrophyllus D. Don, hal=P. hallii Kirk, ner=P. neriifolius D. Don, sal=P. salignus D. Don

Table III. Structure of the Lactones in Subgroup C and their Sources



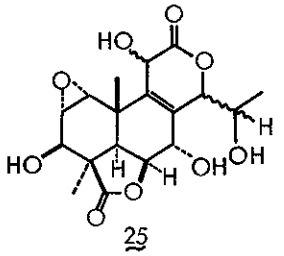
No	compound names	m.p.*1	sources*2	R 1	2	3	15	16	17
21	nagilactone F[8]	225-6°	nag, sel mac, lam				H	CH_3	CH_3
22	ponalactone A[15]	266-9°d.	nak		-O-	OH	H	CH_3	CH_3
23	podolactone E[16]	261-2°	ner		-O-		CH_2=C		H
24	nubilactone A[17]	288-90°	nub		-O-	OH	H, CH_3		CO_2Me

*1 d: with decomposition

*2 nag=P. nagi Zoll. et Moritzi, mac=P. macrophyllus D. Don, sel=P. sellowii Klotzsch, lam=P. lambertii Klotzsch, nak=P. nakaii Hay, nub=P. nubigena Lindley, ner=P. neriifolius D. Don

These dilactones can conveniently be classified in three subgroups depending on the part structure in δ-lactone part: i) A with α-pyrone (Table I), ii) B with dihydro-α-pyrone (Table II) and iii) C having dihydro-α-pyrone with heteroannularly extended unsaturation (Table III). Although carbocycles in these compounds are generally extensively oxygenated, a few common features are noted. In group A with only two exceptions, C_7 has β-hydroxyl group and ring A has at least one hydroxyl group. The presence of 1α,2α-oxido ring with 3β-hydroxyl group observed in this group is also common in the subgroups B and C. The more characteristic feature in the subgroup B is almost ubiquitous appearance of 7α,8α-oxido linkage and occurrence of 1α,2α-oxido

linkage. Only in this group a double bond appears in ring A. Where C_{14} is tetravalent (subgroup B and C), H_{14} has invariably β-configuration. A large variety of the side chain at C_{14} is found, although they are biogenetically related to isopropyl group in totarol: In subgroup A, isopropyl group is intact with two exceptions, while in groups B and C, oxidation occurs frequently at C_{15} and C_{16}, and in several cases one of the isopropyl methyls is missing to result in a vinyl or ethyl group. Only



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exception for this classification is inumakilactone C, 25[11], which posseses β,γ-unsaturated α-hydroxy-δ-lactone system. In a few cases, these compounds (11, 22) have been isolated as O-β-glucoside.[6,15]

Spectroscopic Properties

The rather common general substitution pattern makes the structural investigation easy when the spectra of new compounds were compared with those already reported. In fact, apart from a few compounds investigated at early stage, many structure elucidations relied heavily on the previous work. The following spectral data will help the future study in this field.

UV Spectra

Only chromophor giving UV maximum originates from ring C. As shown in Table IV, each subgroup is easily recognizable; subgroup A by λ_{max}^{MeOH} 300-305 nm (ε 5200-5600) and its bathochromic shift in alkaline solution ($\lambda_{max}^{NaOH-MeOH}$ 370 nm), subgroup

B by λ_{max} 217-221 nm (ε 7300-13500), subgroup C by λ_{max} 257-263 nm (ε 12000-15000) and 25 by no maximum.

Table IV. UV Maxima of Lactones

Subgroup	Cpd No.	λ_{max}^{ROH} nm (ε)	Subgroup	Cpd No.	λ_{max}^{ROH} nm (ε)
A	1	221, 300	B	14	221 (8200)
	2	300 (5200)		15	218 (12500)
	3	300		17	218 (9750)
	4	305		18	218 (12500)
	6	300		19	217 (13500)
	7	300 (5600)			
			C	21	262 (11800)
B	9	219 (12900)		22	262 (12500)
	10	218 (13000)		23	257 (14400)
	11	220 (11000)		24	263 (23000)
	13	220 (7300)		25	262 (15000)

IR Spectra

The most characteristic features common in the IR spectra of the lactones are 1) very strong carbonyl bands due to γ-lactone (at 1760-1780 cm^{-1}) and δ-lactone (at 1700-1730 cm^{-1}), and 2) a weak band due to C=C conjugated with C=O (at 1610-1650 cm^{-1}). The latter two bands range, respectively, at 1685-1740 cm^{-1} and 1620-1640 cm^{-1} for subgroup A, at 1705-1733 cm^{-1} and 1640-1650 cm^{-1} for subgroup B, and at 1700-1720 cm^{-1} and 1608-1643 cm^{-1} for subgroup C. In addition to these carbonyl bands, strong absorptions commonly or often observed are naturally due to the C-O and OH stretching vibrations. Although shift of γ-lactone carbonyl frequency upon the acetylation of 3β-OH was claimed to indicate the hydrogen bonding and therefore the cis orientation of the two groups (for 3,[4b] 4,[4b] 2[8] and 11[10]), this shift has to be taken as an evidence with caution, because other compounds with no hydroxyl at C-3, (2[4a]

and, to some extent 15[12]), exhibit similar shifts. IR data seem rather deviated and much can not be treated as a whole without further systematic study.

NMR Spectra

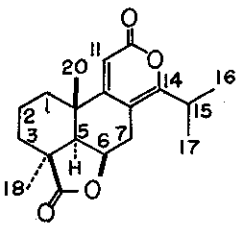
Nmr spectra of these dilactones are in principle quite informative and therefore of great diagnostic value for this type of lactones, as almost all carbon atoms are differently substituted. However, many of these dilactones have poor solubility so that the choice of the solvent is limited to pyridine or DMSO, and even if these solvents are employed some of the signals can not be analysed directly. Both problems can however be overcome by converting them to the appropriate derivatives. In most cases, simple acetylation suffices. The resulted acetates are soluble in most organic solvents so that overlapped signals in a solvent can be separated by the use of another or a mixed solvent to allow analysis.

Spectra thus obtained are usually spread over the range of δ 1.00 ppm and δ 7.00 ppm and well separated from each other so that it is not difficult to perform nmdr experiments. It is even possible nowadays to figure out the structure by simple inspection as the substitution pattern in these dilactones are well recognized. Structural studies on many members isolated later actually depended largely on the nmr assignment of those investigated earlier; thus spectra of nagilactones (2, 3, 4[4]) and inumakilactones (11,[11] 13[10]) served as the reference in many cases.

Nmr spectra of these dilactones are conveniently classified following the structural types. Tables V, VI and VII carry all reported valued for original lactones and/or their acetates. In the following discussion chemical shifts are referred to the pyridine solution in which the spectra of most of the natural lactones were measured.

Nmr spectra clearly exhibit two singlet methyls in all cases. The C_{20} methyl

Table V. NMR Spectra of Lactones (Subgroup A)



Compd No.	Solv.	H_1	H_2	H_3	H_5	H_6	H_7	H_{11}	H_{15}	H_{16}	H_{17}	Me_{18}	Me_{20}
1	Py-d_5	2.52m 1.60m	2.08m 1.94m	4.14t (6)	1.84d (6)	5.14dd (6, 8)	5.64d (8)	7.32s	3.48m	1.23d (6)	1.33d (6)	1.98s	1.32s
2	DMSO	3.79t (6)			1.78d (6)	4.90dd (6, 10)	5.15d (10)	6.48s	3.21m	1.16d (6)	1.18d (6)	1.27s	1.20s
3	Py-d_5	3.72d (4.2)	3.5-3.7	4.68d (6)	2.07d (8)	5.10dd (8, 9)	5.70d (9)	6.69s	3.2-3.6	1.26d (6.5)	1.33d (6.5)	1.50s	2.05s
4	$CDCl_3$	3.63d (4)	3.48dd (4, 6)	4.48d (6)	1.91d (6.5)	5.0tt (6.5, 7, 10)	3.46dd (10, 16) 2.80dd (7, 16)	6.35s	2.64q (7.5)	1.18t (7.5)		1.43s	1.26s
6	Py-d_5	4.29d (3)	4.29td (3, 5)		1.90d (6.5)	5.20dd (6.5, 7.5)	5.65d (7.5)	6.95s	3.50m (6)	1.27d (6)	1.32d (6)	1.92s	1.45s
7-Ac_3	$CDCl_3$	5.05m			1.87d (5)	5.05m	6.25d (10)	5.90s	3.22m	1.25d (8)	4.15m	1.45s	1.30s

Table VI. NMR Spectra of Lactones (Subgroup B) (numbering is same as in Table V)

Cpd No.	Solv.	H_1	H_2	H_3	H_5	H_6	H_7	H_{11}	H_{14}	H_{15}	H_{16}	H_{17}	Me_{18}	Me_{20}
9-Ac	$CDCl_3$			5.00m	1.94d (3.5)	4.96dd (1.5, 3.5)	3.98d (1.5)	5.98s	4.46d (3.5)					
10	$CDCl_3$				1.84d (4.4)	4.95dd (4.4, 1.6)	3.94d (1.6)	5.99s						
11	Py-d_5	3.62d (4)	3.51dd (4,6)	4.65d (6)	2.13d (5.5)	5.08	5.08	6.73s	4.72d (8.5)	4.31m	1.56d (6.5)		1.53s	1.40s
11-Ac_2	Py-d_5	3.73d (4)	3.62dd (6.2,4)	5.76d (6.2)	2.27d (5)	5.13dd (5,1.5)	4.47d (1.5)	6.89s	5.27	5.27	1.53d (6.4)			
12	Py-d_5	3.28d (4)	3.39sx (4,2,2)	2.25dd (2,15.5) 1.72dd (2,15.5)	1.82d (5)	5.15dd (1.5,5)	3.9d (1.5)	6.2s	5.26d (7)	5.95spt (7,8, 17.5)	5.38dd (10,1.5)		1.38s	1.31s
13	Py-d_5	3.63d (4)	3.51dd (4,6.5)	4.67d (6.5)	2.16d (5.2)	5.10dd (1.2, 5.2)	3.95d (1.2)	6.78s	5.39d (6.5)	5.3-6.0			1.51s	1.41s
14-Ac	Py-d_5	3.28d (4)	3.39sx (4,2,2)	2.21dd (2,16) 1.74dd (2,16)	1.82d (4)	5.15dd (1.5,4)	4.41d (1.5)	6.16s	4.82d (4)	1.8-2.4 m		4.2-4.8 m	1.44s	1.37s
15	Py-d_5	3.23d (4.5)	3.36m (4.5, 1,2)	2.17dd (2,15) 1.70dd (1,15)	1.77d (5)	5.07dd (5,<1)	5.18d (<1)	6.11s	4.97s		4.25d (11.5)	1.80s	1.45s	1.41s
16	Py-d_5	3.59d (4)	3.46dd (4,6)	4.59d (6)	2.06d (5)	4.99dd (5,<1)	5.23d (<1)	6.69s	5.05s		4.17d (12) 4.45d (12)	1.78s	1.53s	1.34s

Table VI. NMR Spectra of Lactones (Subgroup B) (continued)

Cpd. No.	Solv.	H_1	H_2	H_3	H_5	H_6	H_7	H_{11}	H_{14}	H_{16}	H_{17}	Me_{18}	Me_{20}	others
17	Py-d_5	3.08d (1.9)	3.30m		1.61d (5.3)	4.86m	1.5-1.9	5.68d (2.5)	4.23dd (2.4, 11.5)		1.25d (7)	1.40s	1.24s	3.60 (CO_2Me)
18	Py-d_5	3.26d (4)	3.37m (4, 2, 1)	2.21dd (2, 15) 1.73dd (1, 15)	1.80d (5)	5.03dd (5, 1.2)	5.23d (1.2)	6.19s	4.83s	3.39d (14.0) 3.76d (14.0)	1.85s	1.43s	1.39s	2.66 (SOMe)
19	Py-d_5	5.88d (10.5)	5.75m (10.5, 3.5, 1)	2.03brs (2H)	2.05d (5)	5.06dd (5, 1.3)	5.25d (1.3)	6.19s	4.89s	3.40d (13.5) 3.76d (13.5)	1.86s	1.29s	1.15s	2.67 (SOMe)

Table VII. NMR Spectra of Lactones (Subgroup C and Inumakilactone C) (numbering is same as in Table V)

Cpd No.	Solv.	H_1	H_2	H_3	H_5	H_6	H_7	H_{11}	H_{14}	H_{15}	H_{16}	H_{17}	Me_{18}	Me_{20}	others
21	$CDCl_3$				1.93d (4.5)	5.04td (2,4.5, 4.5)	6.18dt (2,2, 4.5)	5.74d (2)	4.85q (2)						
22	DMSO	3.54d (4)	3.43dd (4,6)	4.43d (6)	2.19d (5)	5.07dt (5,5, 1.7)	6.28dt (5,2,2)	6.14d (2)	4.95 ddd (1.7,2, 2.5)	2.32 d,sep. (2.5, 6.5)	0.96d (6.5)	1.17d (6.5)	1.19s	1.46s	
23	Py-d_5	3.69d (4.5)	3.56dd (4.5,6)	4.69d (6)	2.11d (5.5)	5.06m (5.5,4, 1.7)	6.24m (4,1.8, 2)	6.51d (1.8)	5.56* (7.6, 0.6, 0.7)	6.09* (7.6, 10.6, 17.3)	5.48* (0.6,10.6,1.3) 5.53* (0.7,17.3,1.3)		1.47s	1.52s	
24-Ac	Py-d_5	3.46	3.46	5.42d (5.1)	2.12d (5)	4.85 ddd (5,4,0.3)	6.09	6.10d (1.5)	5.24 br.d (6)	3.15d,q (7,3.9)	1.38d (7)		1.53s	1.13s	3.66 (CO_2Me)
25-Ac_4	$CDCl_3$	3.52d (4)	3.45dd (4,6)	5.47m	2.10d (5)	4.89 ddd (5,1.7, 5)	6.23 ddd (1.7, 1.7,5)	6.17d (1.7)	5.29 ddd (1.7, 1.7,4)	5.17m	1.34d (6.2)		1.56s	1.17s	

* computer analysis

signal usually appears at higher field than the C_{18} methyl. Other signals invariably present at higher field (1.8-2.1 ppm) is due to H_5 (d, $J_{5,6}$=~5 Hz). Although protons on ring A are unidentified in most unsubstituted cases, H_1 and H_2 are recognizable at δ 3.2-3.7 ppm ($J_{1,2}$=~4 Hz) when an α-oxido linkage is formed between C_1 and C_2. Another oxido proton H_7 in subgroup B appears as a characteristic nallowly-spaced doublet ($J_{6,7}$= <1.5 Hz) at somewhat lower field than H_1 and H_2. The carbinyl proton signal H_3 is in the same region ($J_{2,3}$=~6 Hz) but can be distinguished by its acetylation shift. Another carbinyl proton H_7 in the subgroup A appears in lower field (δ 5.6-5.7 ppm). Magnetic anisotropy of α-pyrone ring may be responsible to the unusual chemical shift. Coupling constant ($J_{6,7}$=7.5-10 Hz) of this proton is larger than that of H_3.

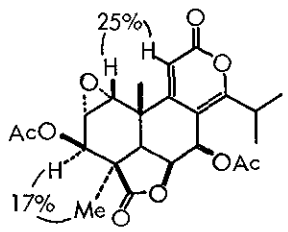
The γ-lactone proton H_6 in subgroups A and B appears as a quartet at δ 5.0-5.2 ppm; splitting pattern differs depending on the substitution on C_7. H_6 in subgroup A with 7β-OH group has larger coupling constants ($J_{6,7}$=7-10 Hz) than those ($J_{6,7}$= <1.5 Hz) in subgroup B with 7α, 8α-oxido group. H_6 in subgroup C usually has an additional long-range coupling with H_{11} (J=~2 Hz).

Signal due to H_{11} usually appears at lowest field as a singlet for subgroups A and B and a narrowly-spaced doublet for subgroup C. Its chemical shift also has diagnostic value; it appears at 6.7-7.3 ppm for subgroup A, and at 6.1-6.7 ppm for subgroup B, showing a larger anisotropic effect of α-pyrone in the former group. In subgroup C, H_{11} appears at 6.1-6.5 ppm, but another low field vinyl proton (H_7) with three coupling constants is observed. H_7 is coupled with H_{14} (J=~2 Hz) in addition to H_6 (J=~5 Hz) and H_{11} (J=~2 Hz). For inumakilactone C 25, H_7 also has couplings with H_{11} (J=1.7 Hz) and H_{14} (J=1.7 Hz) in addition to that (J=1.5 Hz) with H_6. Vinylic

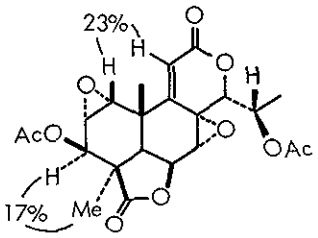
protons in ring A appears at δ 5.7-5.9 ppm, in between H_{11} and other protons, and can be recognized without much difficulty.

Signals due to the side-chain protons and H_{14} have to be analysed separately because these are blocked by a quaternary carbon from the rest of proton sequence. Analysis is however rather straightforward. It is noted that where isopropyl group is present at C_{14}, the methyl signal always appears as two sets of doublet probably due to the magnetic non-equivalence. Thus, nmr analysis is quite informative in elucidating structure of these lactones.

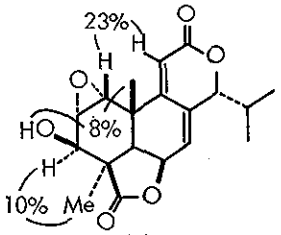
One special feature of nmr used in these studies are nuclear Overhauser effect. The effect was observed in derivatives of all types of compounds between 1) $H_{1\beta}$ and H_{11} (13-25%), 2) $H_{3\alpha}$ and Me_{19} (10-17%) and 3) 3β-OH and Me_{20} (8% in DMSO). Typical examples in each subgroup are shown.



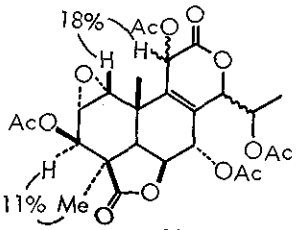
3-Ac_2 [4b]



11-Ac_2 [10]



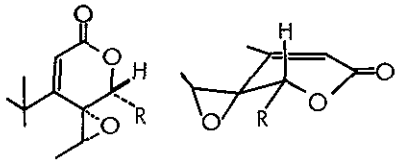
22 [15]



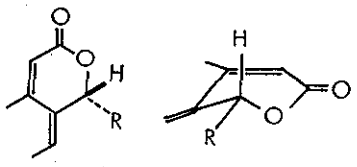
25-Ac_4 [11]

Chiroptical Properties

In the present series of investigations, circular dichroism (CD) and optical rotatory dispersion (ORD) have been used effectively in determining conformation, and relative and absolute configurations. Strong negative Cotton effect ($[\theta]_{259\text{-}263}$= -15000～-22200 or $[\theta]_{262\text{-}265}$= -31700～-51500) observed in subgroups B and C, respectively, suggests the conformation shown below of ring C. ORD has been recorded also on subgroup A: Nagilactones A and B exhibit positive Cotton effect ($[\Phi]^{320}_{max}$ - $[\Phi]^{275}_{min}$= +3850～ +4280)[4c]. Inumakilactone C 25 shows a negative Cotton effect ($[\theta]_{225.5}$= -9350).[11]

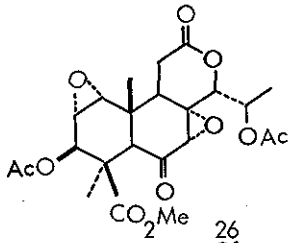


Subgroup B

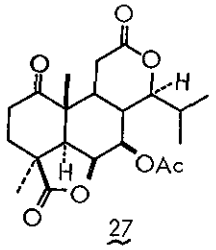


Subgroup C

The 7,8-oxido-6-one 26 derived from 11, showed a negative Cotton effect at n-π* transition[10], from which absolute configuration of 11 was deduced on the basis of known general rule[18] on such α-epoxy ketones.



26



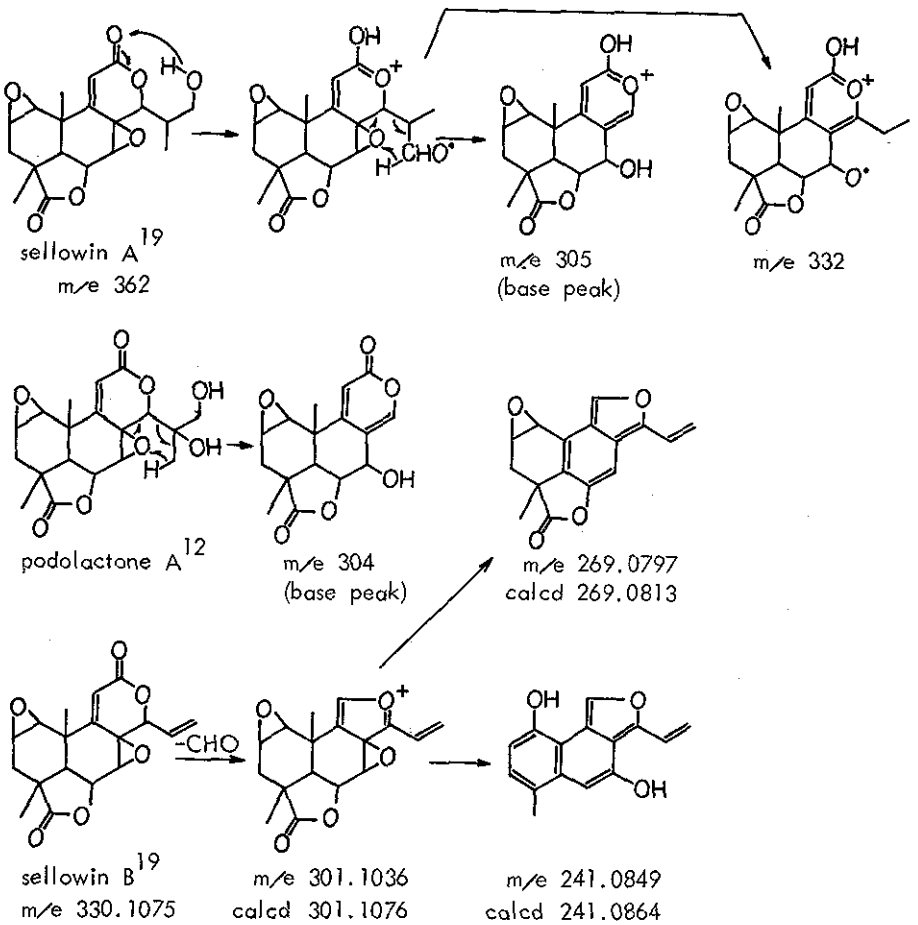
27

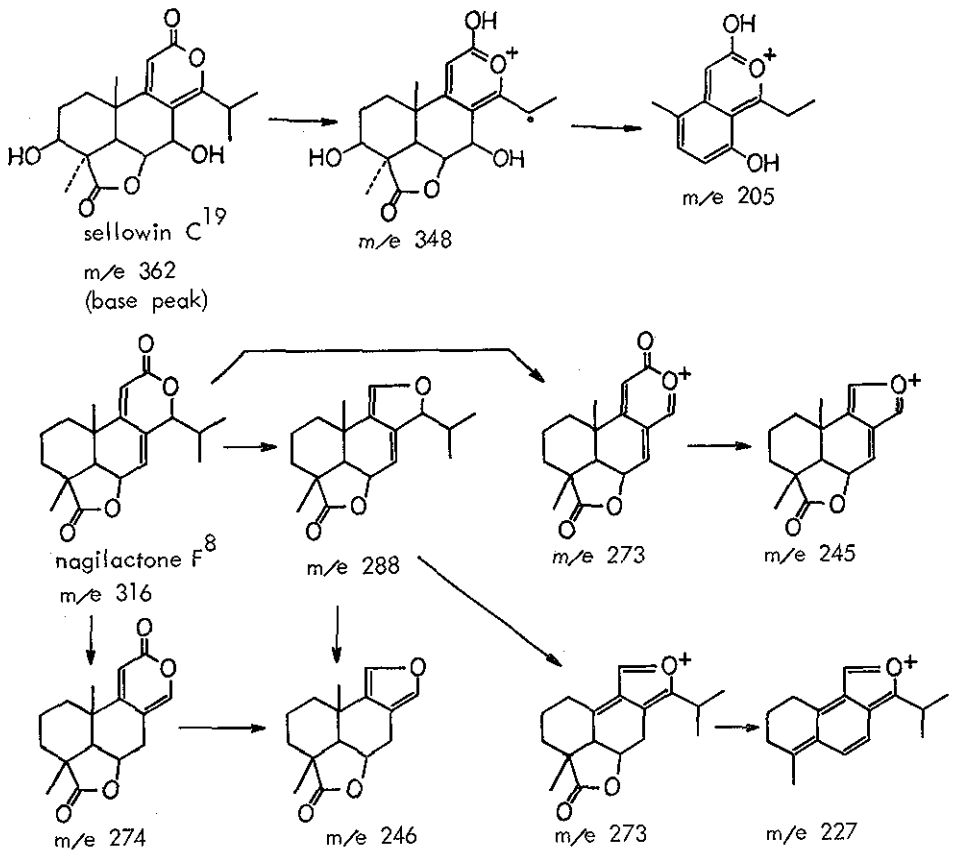
The 1,2-epoxy-3-ones derived from 3[4b], 4[4b], 11[10] and 13[11] exhibited negative n-π* Cotton effects ($[\theta]_{334\text{-}5}$= -2170～-3210, $[\theta]_{325}$= -2410～-3230). The Cotton effect in these cases does not follow the "reverse octant rule", probably due to a large contribution of the rest of the molecule. The [θ] values observed are very small.

The 1-one 27 derived from 2 has a large Cotton effect ($[\theta]_{297}$= -4800).[4c]

Mass Spectrometry

Although no systematic study on these dilactones has been appeared, fragmentation processes in subgroups A, B and C were discussed in the courses of the structure elucidation. In all cases, fragmentation seems to occur first at ring C and side chain, and then at γ-lactone ring. The proposed fragmentation processes are shown below.





X-ray Crystallography

This powerful method was applied only to a few cases in this interesting group of compounds. Configuration of nagilactone A (subgroup A) was established by X-ray analysis of its 3,7-diacetate[4c]. The compound has its ring A in distorted chair and ring B in distorted boat form, while ring C is nearly planer. γ-Lactone ring forms a C(5) envelope. The structure elucidation of podolide (subgroup B) heavily relied on this technique[9]. In this compound, the γ-lactone ring is again a C(5) envelope. Rings A and B have slightly distorted boat conformation, while the ring C has slightly

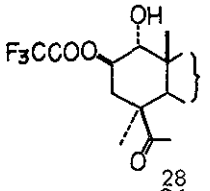
distorted pseudochair conformation. X-ray study on podolactone A has also been carried out.[20]

Chemical Reactions

Since the physical methods are extensively used in structure elucidation, only a few chemical transformations have been reported on these dilactones. The functional groups in these compounds show rather unusual stability. This may due to the solubility problem mentioned earlier and the stereochemical factors. In this section the reactions of the each functional group are discussed.

Epoxy Linkages

Epoxide rings in the dilactones have unusual stability toward acids. Thus, the oxide in nagilactone C 3 is resistant to trifluoroacetic acid.[19] However, the similar ring in sellowins A and B (14 and 12) was attacked by the same acid to give 1α-hydroxy-2β-trifluoroacetoxy derivative 28.[19] Inumakilactone A 11 is also attacked by acids (HBr, HCl and H_2SO_4), but the products have 1β-halo (or hydroxy)-2α-hydroxy structure 29.[6] Since the ring A is known to exist in twisted boat conformation which



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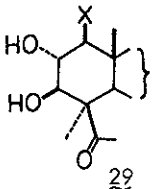

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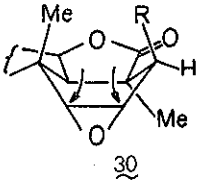


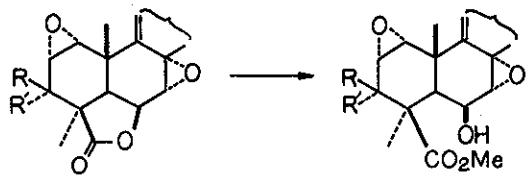
30

would allow formal diaxial opening in either case, the positional difference in the nucleophilic attack may be due to 1) steric hindrance on β-side where nucleophile approaches to the cationic center, and 2) interaction of 3β-hydroxyl group with nucleophile (e.g. hydrogen-bonding) or with C_2 (e.g. decrease of the net charge) (Cf. 30). Ring A epoxide in 3 can be removed by reduction with Cr^{++} or Zn-Cu, by

which sellowin C 1 was obtained.[9] 6,7-Epoxide ring in subgroup B resists to the acidic ring opening.[6,19]

γ-Lactone

Both lactone parts in subgroup B can be opened by alkali. To open γ-lactone preferentially, sodium carbonate can be used in absolute methanol to result in the corresponding hydroxy methyl ester 31.[10,21]



R=OH, R'=H

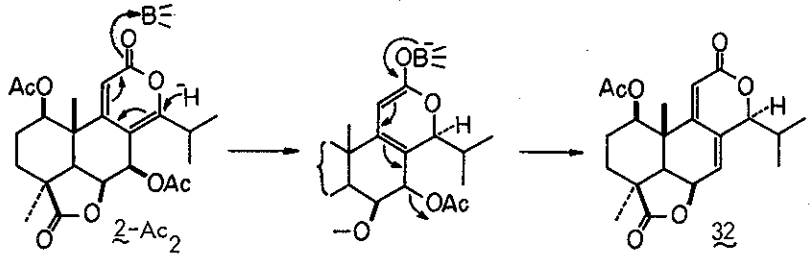
R, R'=O

31

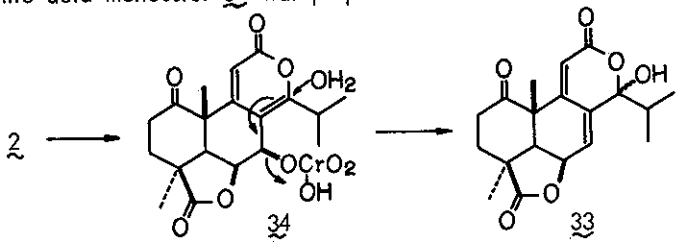
δ-Lactone

Although both lactone parts in subgroup B can be cleaved by alkali, δ-lactone opens preferentially, as was witnessed by ir spectrum (ν 1760 cm^{-1}) of pottasium salt of 11, and recloses quantitatively upon acidification.[22]

In subgroup A, interesting reactions were observed for nagilactone A, 2. On borohydride reduction of its diacetate, 2 yielded a doubly unsaturated chromophor, 32, identical with the one found in subgroup C, the reaction suggestive of the biogenesis of the latter group.[4a] The reaction performed on nagilactone C 3 yielded 14-epi-ponalactone A.[15] The following reaction mechanism was proposed.



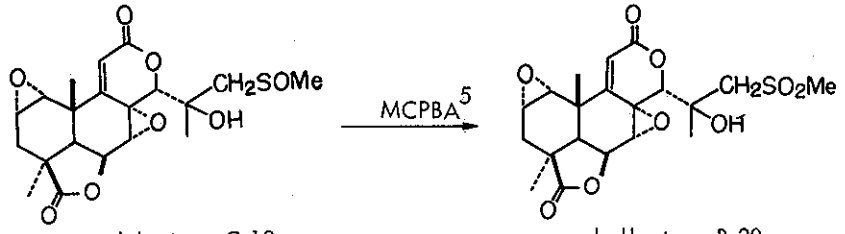
Chromic acid oxidation of 2 is also abnormal: Though 1β-OH behaves normally, 7β-OH is resistant to the oxidation and rearranges to C_{14} position giving 33[4a]. Chromic acid monoester 34 was proposed to be the intermediate.



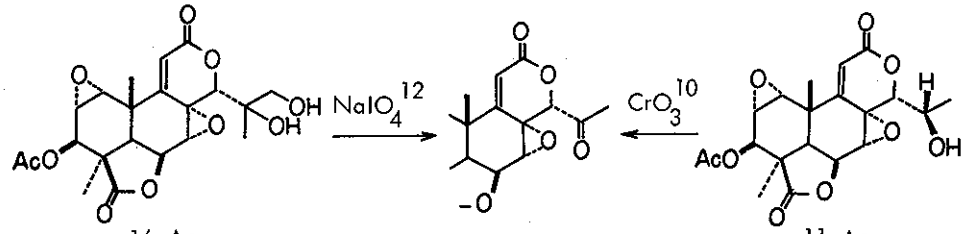
Both reactions may be caused by the steric hindrance at C_8 and C_7 of the crucial intermediates.

Side Chain

Some standard reactions applied to modify side chain of the dilactones failed.[14,19] The followings are the correlation reactions which lead to the structure elucidation of the newly isolated members.



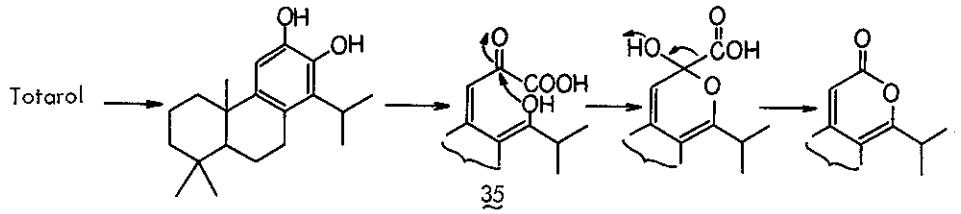
podolactone C 18 hallactone B 20



16-Ac 11-Ac

Biogenesis

The unique and common carbon skeletons observed in this group of dilactones are interesting also from the biogenetic point of view. It is clear that the carbon skeletons are derived from totarol or totarol carboxylic acid, which appear in Podocarpus as congenors. The formation of ring C for subgroup A was suggested by Hayashi, et al[4a] as shown below.



35

Hydroxylation of totarol and subsequent meta-pyrocatechase type fission would lead to the diketo carboxylic acid 35 from which α-pyrone would form by further oxidative decarboxylation. This scheme can be extended a little further to explain the biogenesis of the other subgroups.

Hayashi's conversion of nagilactone A diacetate (borohydride reduction and chromic acid oxidation) to nagilactone F derivative 32[4a] described earlier constitutes the correlation of subgroup A with C, although the reduction afforded 14-epi product.[15] An epoxidation of the double bond in subgroup B from less-hindered side would result in the formation of epoxy αβ-unsaturated δ-lactone, the characteristic part structure in subgroup B. Thus, in nature, these compounds can be envisaged to form in the order of subgroups A, C and B. In accord with this hypothesis, modification of the side chain occurs more extensively following the same order.

Biological Activities

Podocarpus macrophyllus has long been known in Japan for its termite resistance.

Recent investigation[23] disclosed that the termiticidal activity is entirely due to inumakilactone A and another unidentified compound of similar structure, the former being more active. Their activities appear very slowly (7 days after administration). P. nagi is easily attacked by termite. This may be due to the abundance of mannose and galactose in the wood.[24a] Nagilactone C, hallactones A and B, podolactones A, C and E, and sellowin A are known to be toxic to housefly larvae.[4d,5,24b]

Podolide 10 is the first dilactone reported to have anti-tumor activity in vivo against P-388 leukemia in mice and cytotoxicity in vitro towards cells derived from both human carcinoma of nasopharynx (KB) and P-388 murine leukemia.[9] Recent study also revealed a strong cytotoxicity and antileukemia activity of nagilactones, 3, 4, 6 and 9.[25] All of them exhibited the IC_{50} values of 10^{-3} - 10^{-4} mM against the cell culture of the former cells, 4 and 9 being somewhat stronger. 3 and 9 are effective toward P-388 murine leukemia (effective dose: 20mg/kg/day).

By far the more popular biological effect of the dilactones is the growth inhibitory activity. After the first recognition of strong activity on podolactones[12], many of the members have been found to show strong inhibition of expansion and mitosis of plant cells. *Systematic studies*[26,27] *revealed the evaluation of their activities* (Table VIII).

As shown in Table VIII, inhibitory effect of lactones agrees each other fairly well in two systems examined. Although activity is quite different from a lactone to another, there appears not much difference in activity between each subgroups. More characteristic feature is that the fewer the oxygen functions in the side chain, the stronger the activity; thus nagilactones D and F (4, 21), inumakilactone B (13), and podolactone E (23), are the most active compounds. 23 is still active (82% of

Table VIII. Inhibitory Activity of Lactones

Compd. No.	Hook segment[26] *1 % of control growth at 10^{-6}M	10^{-5}M	Avena coleoptile segment[27] conc. of lactones ($\times10^{-7}$M) for 50% growth inhibition*2 IAA: 0.3 ppm	IAA: 0.03 ppm
Subgroup A				
6		81	700	700 $\times10^{-7}$M
2		79	500	700
3	89	53	600	400
4	72	15	30	20
Subgroup B				
18	100	70		
19	98	60		
16	82	60		
9			50	10
15	88	37		
11	66	34	30	10
13	47	10	20	10
Subgroup C				
22			30	10
23	38	0	1	1
Inumakilactone C 25	102 (at 5×10^{-6}M)		inactive	inactive

*1 Etiolated dwarf peas

*2 Competitive inhibition in indoleacetic acid (IAA)-induced elongation at given IAA concentrations

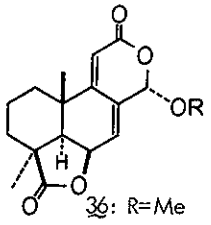
control growth) at 10^{-7}M in hook segment test, which is said to be less sensitive than that with etiolated Avena coleoptile sections. More active compounds have the 1,2-epoxide ring. Although some of the simple derivatives such as acetates of 11, 13 and 23 are still active, almost all modifications, such as hydrogenation of ring C or B, hydrolysis of γ-lactone (31), epimerization of H_{14} (32) and other transformations which cause change in the conformation of ring A, result in a lowering or disappearance of activity. It is particularly interesting that nagilactones A~D (subgroup A) show promoting activity at lower concentration (10^{-7}M - 10^{-8}M), while those (2 and 21) belong to the other subgroups exhibit no such a reversal in activity.

Apart from these structure-activity relationship studies, a few other compounds are reported to have the similar growth inhibitory activity. Those include inumakilactone A 15-β-glucoside,[6] ponalactone A (22) and its 3β-glucoside[15] and sellowin A (14).[3]

Tetranorditerpene Dilactones from Acrostalagmus

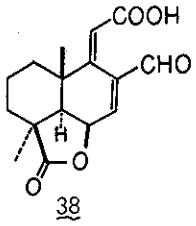
Although not of plant origin, a few metabolites isolated from unidentified Acrostalagmus species have very close similarity in structure and biological activity with the dilactones already discussed, and therefore are reviewed herein briefly.

The compounds are called LL-Z1271α and γ and have the structures 36 and 37, respectively.[28]



36: R=Me

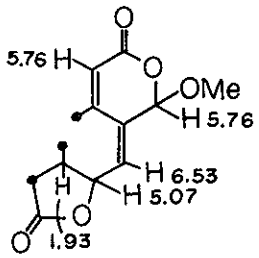
37: R=H



38

They exhibit the characteristic spectral features of subgroup C of Podocarpus dilactones: Uv maximum at 257 nm (ε 13500), ir bands at 1775-80 cm^{-1}, 1720-30 cm^{-1} and CD

curve of $[\theta]_{255} = -2.2 \times 10^4$. Nmr spectra with two singlet methyl signals at 1.11 ppm and 1.38 ppm are reported to be very similar with podocarpic acid derivatives. Especially revealing nmr pattern is as follows.



$J_{5,6} = 5.3$ Hz
$J_{6,7} = 4.7$ Hz
$J_{6,14} = 1.0$ Hz
$J_{7,14} = 2.0$ Hz
$J_{7,11} = 1.8$ Hz

These two compounds were chemically correlated. 37 appears to exist as open aldehydocarboxylic acid 38 in solid state (ν^{KBr} 1780, 1680 cm^{-1}).

Biogenetically these compounds are derived from labdane type diterpene as was established by a tracer experiment.[29] LL-Z1271β, a possible biogenetic precursor, was also isolated.[30] Compound 36 exhibits antifungal activity both *in vitro* and *in vivo* against several experimental fungal infection[28] and significant plant growth inhibition.[27,29] Total synthesis of these compounds has been performed by two groups.[31]

Concluding Remarks

In the past 7 years, about a seventh of the *Podocarpus* species distributed on the earth has been investigated. In view of the uniformity of the carbon skeleton and substitution pattern, the future study will greatly be facilitated by the achievement in the past. However, continued effort in the future may result in the isolation of new members with biological as well as chemical interest. This effort should also contribute to the taxonomy of this genus.

Synthetic effort should also be worthwhile, because, excepting those from *Acrostalagmus*, none of these dilactones has been synthesized and the members with

simpler functionality is known to have stronger biological activities. Even synthesis of some simple analogs may have some practical values.

At the same time, further biological study is certainly needed to evaluate usefulness of this type of natural products and eventually establish their biological functions.[32]

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32 Possible functions of these dilactones are discussed by Brown and Sánchez.[5b]

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