DITERPENOIDS OF *ISODON* AND *TEUCRIUM* PLANTS†

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The Chemistry on diterpenoids of the plants belonging to the genera *Isodon* and *Teucrium* (Labiatae) has recently been developed. This review deals mainly with our research works concerning isolation and characterization of new diterpenoids, their reaction and interconversion, synthesis, biosynthesis, and biological activity.

1 Introduction

The genera *Isodon* and *Teucrium* belong to Labiatae. Since 1963, Fujita and co-workers have investigated the diterpenoids of *Isodon trichocarpus* Kudo and *I. japonicus* Hara. First, a chemical conversion of the major bitter diterpenoid enmein (1) into *ent*-kaurane (2) was carried out to provide a chemical evidence for the structure and absolute configuration of enmein (1).[1] Then, many kinds of diterpenoids have been isolated and their structures elucidated. From *I. japonicus* were isolated the pure enmein, which was not contaminated by dihydroenmein, and enmein 3-acetate (3).[2] From *I. trichocarpus* was isolated trichokaurin (4).[3]

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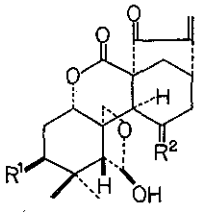
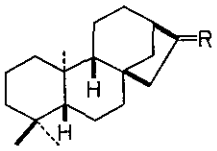
† This paper is dedicated to Dr. Ken'ichi Takeda on the occasion of the 70th anniversary of his birth.

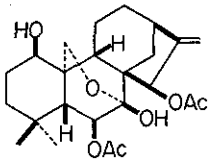
Isodocarpin (5),[4] nodosin (6),[3a,5] isodotricin (7),[4a,6‡] oridonin (8),[4a,7] and trichodonin (9)[8,9] were isolated from both species, and their structures were elucidated.



(1) R^1=OH, R^2=H_2
(3) R^1=OAc, R^2=H_2
(5) R^1=H, R^2=H_2
(6) R^1=H, R^2=α-H,β-OH
(21) R^1=H, R^2=α-OH,β-H



(2) R=α-H,β-Me
(10) R=CH_2



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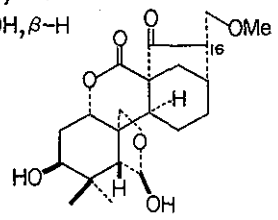

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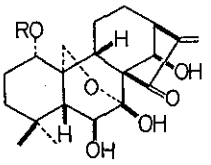
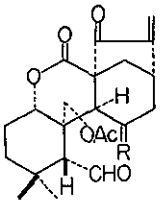


(8) R=H
(28) R=Ac



(9) R=α-H,β-OH
(20) R=α-OH,β-H

In relation to these investigations, a formal chemical conversion[3d] of trichokaurin (4) into *ent*-16-kaurene (10), atisine (11), garryine (12), and veatchine (13), the chemical conversion[10] of enmein (1) into *ent*-abietane (14), and the total synthesis[10] of abietane (15) were accomplished. An interesting epimerisation of enmein derivatives (16) and (17) to (18) and (19), respectively, was studied under mild alkaline conditions.[11]

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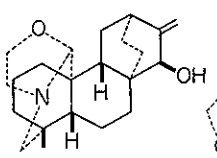
‡ The determination of the stereochemistry of C-16 is described in section 2-1.



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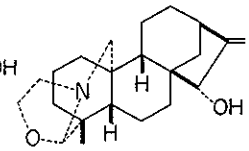

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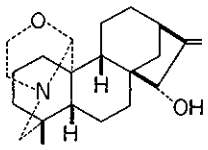

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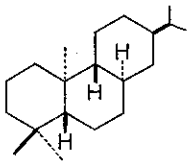

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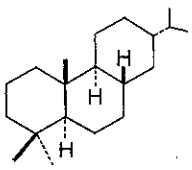

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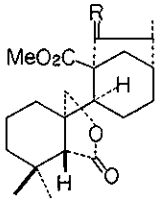
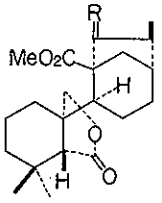


(16) R=α-OH, β-H
(18) R=α-H, β-OH



(17) R=α-H, β-OH
(19) R=α-OH, β-H

The foregoing studies were carried out before 1968 and described in detail by Fujita,[12] one of the authors. In this review, new results obtained to the present time since then are mentioned.

2 Diterpenoids of *Isodon* plants

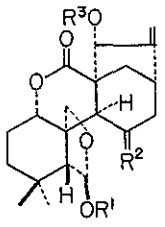
2-1 Isolation and structure determination

2-1-1 Research works in our group

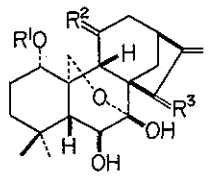
The presence of several other minor diterpenoids in *I. japonicus* was suggested by the thin layer chromatography (t.l.c.) of the plant extract. Among these, the potential biosynthetic intermediates to enmein and the new natural products of interesting structures may be found. In hopes of these matters, a detailed investigation on this plant was carried out to isolate seven diterpenoids, five of which were new. The remaining two were the known isodonal (20) and epinodosin (21), the structures of which

had been determined by Kubota and co-workers.[9] The new diterpenoids were named epinodosinol, sodoponin, isodoacetal, nodosinin, and odonicin.

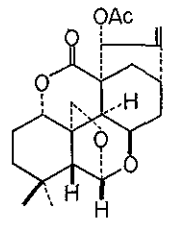
The structures of epinodosinol and sodoponin were determined to be (22) and (23), respectively, on the basis of their correlation to epinodosin (21) and the chemical conversion of sodoponin (23) into epinodosinol (22).[13]



(22) R^1=R^3=H, R^2=α-OH, β-H
(25) R^1=Me, R^2=α-H, β-OH, R^3=Ac



(23) R^1=H, R^2=α-H, β-OAc
R^3=α-H, β-OH
(29) R^1=H, R^2=α-H, β-OH, R^3=O
(33) R^1=Ac, R^2=α-OAc, β-H, R^3=O



(24)

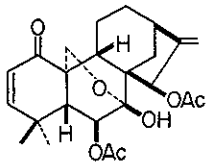
Isodoacetal and nodosinin were shown to have a characteristic acetal ring structure (24) and its ring opening structure (25) formally derived by the attack of methanol, respectively. These structures were determined by their correlation to nodosin (6).[14] Odonicin was shown to be characteristic of the αβ-unsaturated ketone in the ring A and its structure (26) was elucidated on the basis of the chemical evidence in relation to isodocarpin (5) and trichokaurin (4).[14] This compound seems interesting as a potential precursor for the introduction of a hydroxy group to C-3.

The stereochemistry of C-16 in isodotricin (7) was established as S by a detailed investigation of an unequivocal stereoselective transformation of enmein (1) into isodotricin.[6]

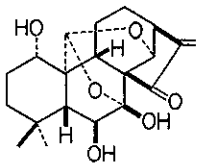
The structure of ponicidin, a minor diterpenoid isolated from *I. japonicus*, was elucidated as (27) on the basis of the chemical and spectroscopic evidence.[15] Especially, the n.m.r. and internuclear double resonance (INDOR) spectra[16] were effectively used in this study and the

absolute configuration was determined by the o.r.d. spectrum of the dihydro-derivative.

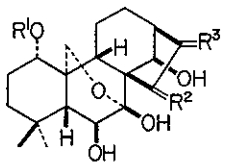
From the ethereal extract of the dried leaves and stems of *Isodon lasiocarpus* (Hayata) Kudo collected in Taiwan were isolated four new diterpenoids, lasiokaurin, lasiodonin, lasiokaurinol, and lasiokaurinin besides the known oridonin (8). The major diterpenoid, lasiokaurin was shown to be identical with oridonin 1-acetate (28) which had been derived from oridonin in the structure determination of the latter, but it was the first time that this compound was found in nature.[17] The structure of lasiodonin was determined as (29) by correlation with sodoponin (23) and chemical conversion into epinodosin (21).[17] The structure of lasiokaurinol was elucidated as (30) by its correlation with oridonin (8) and its formation as a minor product from lasiokaurin (28) by sodium borohydride reduction at 0°.[18] Lasiokaurinin was shown to have a methoxymethyl group at C-16 like isodotricin (7). The latter corresponded to the methanol adduct of enmein (1), but the former was shown to be the 16-epimer (32) of the adduct (31) prepared by the acid catalytic addition of methanol to lasiokaurin (28) by the n.m.r. data of several derivatives and acid catalysed epimerisation of lasiokaurinin (32) into 16-epimer (31).[18]



(26)



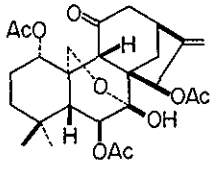
(27)



(30) R^1=Ac, R^2=α-H, β-OH, R^3=CH_2
(31) R^1=Ac, R^2=O, R^3=α-H, β-CH_2OMe
(32) R^1=Ac, R^2=O, R^3=α-CH_2OMe, β-H

2-1-2 Research works in other groups

Kubota *et al.* determined the structures of isodonal (20),[9a,9c] epinodosin (21),[9b,9c] and trichodonin (9)[9b,9c] isolated from *I. japonicus*. After this work, they investigated seven species of the *Isodon* genus. From *I. shikokianus* Kudo, they isolated oridonin (8) and two novel diterpenoids, shikokianin (33)[19] and shikodianidin (34)[20] and determined their structures on the basis of chemical and spectroscopic evidence. From *I. longitubus* Kudo were isolated the known nodosin (6), isodocarpin (5), lasiokaurin (28), and oridonin (8).[21] From *I. Kameba* Okuyama were isolated two new diterpenoids, kamebanin and mebadonin.[21] The structure of mebadonin was determined as (35) by the X-ray diffraction method.[22] From *I. umbrosus* Hara were isolated two new diterpenoids, umbrosin A and B.[21] Their structures were elucidated as (36) and (37), respectively, by correlation with mebadonin (35).[23] They also isolated some diterpenoids from *I. shikokianus* Hara *var. intermedius* Murata, *I. inflexus* Kudo, and *I. effusus* Hara, but the structures of these compounds have not yet been determined.[21] Isolation of diterpenoids, the C-20 of which was not oxidised, is interesting from the viewpoints of biosynthesis and chemotaxonomy.



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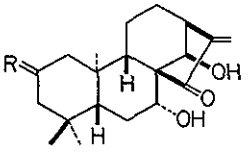
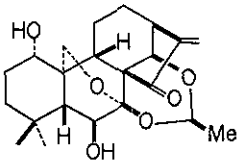


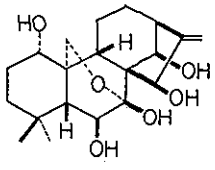
(35) R=α-OH, β-H
(36) R=α-H, β-OH
(37) R=O



(38)

Okamoto *et al.* isolated eleven diterpenoids in addition to enmein (1) from *I. trichocarpus* Kudo, and determined the structures of isodonol,[24]

enmedol,[24] enmenol,[24] enmenin,[25] enmelol,[25] and ememodin.[25] Isodonol and enmenin were proved to be identical with oridonin (8) and trichokaurin (4), respectively. The structure of enmedol (38) was determined by the spectroscopic and chemical evidence that condensation reaction between oridonin and acetaldehyde afforded enmedol as a sole product. Enmenol was assigned structure (39) by correlation with oridonin (8) and the chemical conversion into enmedol (38). The structure of enmelol (40) was determined on the basis of its formation from trichokaurin (4) by treatment with lithium aluminium hydride. Ememodin was proved to be identical with dihydrodehydroenmein (41).



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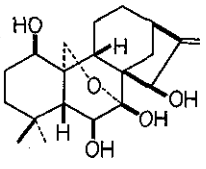

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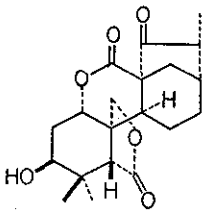


(41)

2-1-3 Conclusion

Thus, the structures of thirty diterpenoids isolated from the *Isodon* plants have been elucidated, including dihydroenmein[26] which was not described above. The authors suggested a biogenetic classification of these diterpenoids.[27]

The mebadonin (35)-type diterpenoids which were not oxidised at C-20 have occurred in *I. Kameba* Okuyama and *I. umbrosus* Hara. From *I. shikokianus* Hara and *I. lasiocarpus* Kudo have been isolated only the poly-oxygenated *ent*-16-kaurene derivatives. *I. longitubus* Kudo, *I. trichocarpus* Kudo, and *I. japonicus* Hara have been shown to contain the *ent*-6,7-seco-16-kaurene derivatives as well as poly-oxygenated *ent*-16-kaurene

derivatives. These observations together with new developments of the *Isodon* diterpenoids chemistry in the future may make chemotaxonomy of these plants possible.

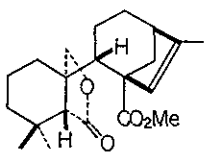
2-2 Chemical conversions

The *Isodon* diterpenoids can generally be classified into two structural groups *i.e.* *ent*-16-kaurene derivatives and *ent*-6,7-seco-16-kaurene derivatives. Biogenetically a suitable kaurene type compound is reasonably regarded as the precursor for a 6,7-secokaurene type compound. In fact, *ent*-16-kaurene was proved to be a precursor for enmein (1), as described in section 2-4. The chemical conversions with the *Isodon* diterpenoids, therefore, often involved the biogenetic 6,7-bond fission or the retro-biogenetic 6,7 carbon-carbon bond formation as the key step. Thus oxidative cleavage by metaperiodate and acyloin condensation were effectively used. The chemical conversions of enmein (1) into *ent*-kaurane (2)[1] and *ent*-abietane (14)[10] described in the previous review [12] are the typical examples of the retro-biogenetic type chemical conversions applying the acyloin condensation. The retro-biogenetic type (2-2-1 ∿ 2-2-3), biogenetic type conversions (2-2-4 ∿ 2-2-7), and other conversions (2-2-8) are successively described.

2-2-1 A formal chemical conversion of enmein into *ent*-16-kaurene, atisine, garryine, and veatchine[28]

Acyloin condensation with the unsaturated lactone ester (42) derived from enmein (1) gave the products (43)-(47). The acyloin (43), after acetylation, was subjected to the photosensitised oxygenation to yield 15α-ol (48), which was subjected to successive acetylation, ozonolysis, and hydrogenolysis (calcium in liquid ammonia) to afford compound (49). This

compound had been converted into *ent*-16-kaurene (10), atisine (11), garryine (12), and veatchine (13),[3c,3d,10] hence this work constituted the chemical conversion of enmein (1) into these diterpenes.



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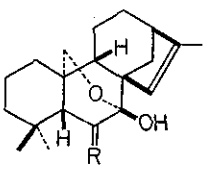
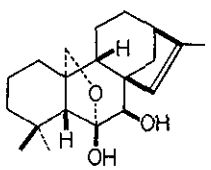


(43) R=α-H, β-OH
(45) R=O



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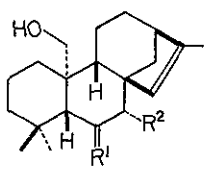
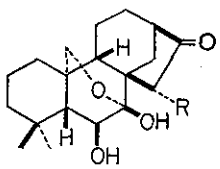
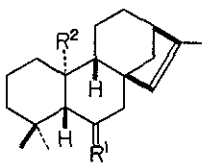


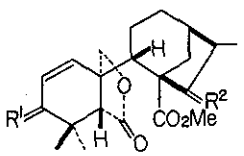
(46) R¹=α-OH, β-H, R²=OH
(47) R¹=α-H, β-OH, R²=H



(48) R=OH
(49) R=H



(50) R¹=H₂, R²=Me
(51) R¹=O, R²=CHO



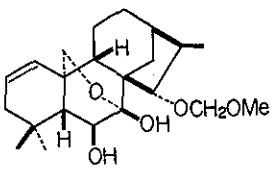
(52) R¹=R²=O
(53) R¹=-SCH₂CH₂S-, R²=O
(54) R¹=H₂, R²=α-OCH₂OMe, β-H

2-2-2 The chemical conversion of enmein into *ent*-15-kaurene and *ent*-16-kaurene[29]

The chemical conversion of diol (47) derived from enmein through the foregoing route into *ent*-16-kaurene (10) and *ent*-15-kaurene (50) was successfully carried out. The diol (47) on Jones oxidation gave 6-oxo-20-al product (51), whose Huang-Minlon reduction (Barton's modification) gave *ent*-15-kaurene (50), *ent*-16-kaurene (10), and *ent*-kaurane (2) in a ratio of 5 : 2 : 3. The Nagata's modification[30] of Wolff-Kishner reduction with dione (51) afforded only *ent*-kaurane (2) unexpectedly. The model experiments were thus carried out and the mechanisms were discussed.[31]

2-2-3 The chemical conversion of enmein into enmelol[32]

The lactone ester (54) was derived from enmein (1) *via* (52), (53) *etc.*, and the acyloin condensation with this compound (54) gave the desired product (55) together with a few minor products. Epoxidation, reductive opening of the epoxide ring by lithium aluminium hydride, 1,6-diacetylation, elimination of the protecting group at 15-ol and subsequent dehydration at C-15 (mesyl chloride in pyridine) afforded compound (56), which on photosensitised oxygenation yielded 15-one (57) as a minor product. Sodium borohydride reduction at 0° followed by treatment with lithium aluminium hydride converted (57) into enmelol (40).



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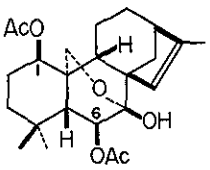
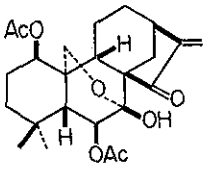


(56)

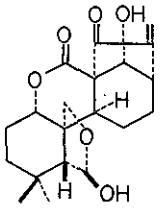


(57)

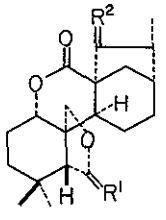
2-2-4 The chemical conversions of oridonin, enmein, nodosin, and trichokaurin into isodocarpin

Oridonin (8) on periodate oxidation gave compound (58), which on successive Jones oxidation, catalytic reduction, mesylation, and hydrogenolysis on Raney nickel at high pressure afforded isodocarpin dehydrotetrahydro-derivative (59).[7b] Tetrahydropyranylation, reduction of the γ-lactone to hemiacetal (lithium aluminium hydride at -25∿-35°), methyl acetalisation and elimination of the protecting group (hydrochloric acid in methanol), and chromic acid oxidation converted (59) into (60) which on bromination at C-16 (NBS and perbenzoic acid in chloroform) and

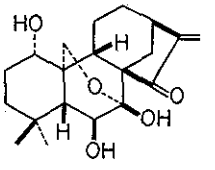
treatment with lithium chloride in dimethylformamide followed by acidic hydrolysis of acetal (aqueous acetic acid) yielded isodocarpin (5).[7c] The compound (60) was derived from isodocarpin dihydro-derivative, which had been derived from enmein (1),[4] nodosin (6);[4b,5] and trichokaurin (4),[3d] hence this work constitutes also accomplishment of the chemical conversions of these natural diterpenes into isodocarpin (5).



(58)



(59) R^1=O, R^2=α-OH, β-H
(60) R^1=α-H, β-OMe, R^2=O



(61) ≡ (147)

2-2-5 The chemical conversion of trichokaurin into isodocarpin *via* a direct pathway[33]

The chemical conversion of trichokaurin (4) into isodocarpin (5) had been accomplished. (See section 2-2-4.) But the second and more biogenetic chemical conversion was successfully tried. Trichokaurin (4) was converted into (61), a potential biosynthetic precursor of isodocarpin, *via* chromic acid oxidation to 1-oxo-derivative, sodium borohydride reduction to 1α-ol, 6,15-dideacetylation (lithium aluminium hydride), and dehydrogenation to C-15 ketone (DDQ). The compound (61) on periodate oxidation gave isodocarpin (5). When sodium borotritiide was used in the foregoing reduction of the 1-oxo-derivative of oridonin, 1β-tritium labelled compound of (61) was obtained. It was incorporated into oridonin by *I. japonicus*, which is described in section 2-4.

2-2-6 The chemical conversion of lasiodonin into epinodosin[17]

In the studies of structure determination of lasiodonin (29), it was converted into epinodosin (21) by the periodate oxidation.

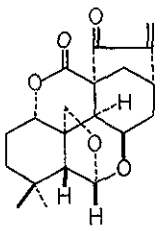
2-2-7 The chemical conversion of sodoponin into epinodosinol[13]

In the studies of structure determination, sodoponin (23) was subjected to periodate oxidation followed by alkaline hydrolysis to yield epinodosinol (22).

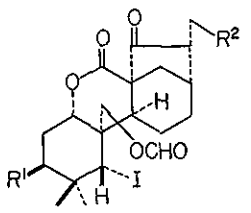
2-2-8 The others

The other chemical interconversions of the natural products are as follows : enmein (1) to *dihydroenmein*, *dihydroenmein* to enmein,[7c] enmein to isodotricin (7),[6] enmein to its 3-acetate (3),[2] and oridonin (8) to lasiokaurin (28).[7c,17]

A biogenesis of nodosin (6) from isodocarpin (5) *via* an isodoacetal (24)-like intermediate (62) was suggested. So the hypoiodite reaction was tried with isodocarpin 16,17-dihydro-derivative, *dihydroenmein* 3-acetate, and isodotricin 3-acetate, but the 5,6-cleaved iodo formate products (63)-(65) were obtained, respectively, instead of the desired (62)-type compounds.[34]



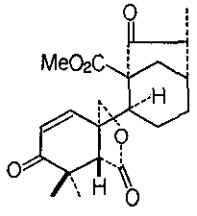
(62)



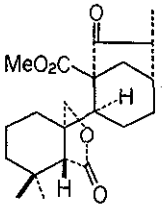
(63) $R^1=R^2=H$
(64) $R^1=OAc$, $R^2=H$
(65) $R^1=OAc$, $R^2=OMe$

Sim *et al.*[35] carried out the X-ray analysis of the bromoacetate of compound (18) prepared by us and provided an evidence for the structure of

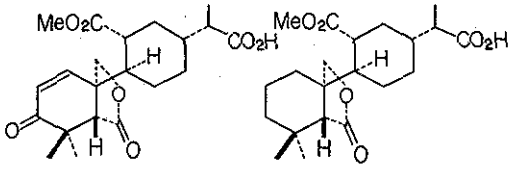
(18). They also showed the chair conformation for the rings A and C of (18). Several epimeric enmein derivatives including compounds (16)-(19) were compared on the thin layer chromatogram, and an interesting regularity was observed between the Rf value and the stereochemistry of their functional groups.[36] Subsequently, the mechanism for the epimerisation of (16) to (18) and other related epimerisation was studied using the deuterio- or tritio-labelled compound of (16). As the result, the retro-aldol type mechanism in which a common stereoelectronic requirement was satisfied in the transition state was suggested.[37] This concept was developed and applied to the mechanisms for the retro-Dieckmman type cleavages of ketones (66) and (67) into caboxylic acids (68) and (69), respectively, an easy epimerisation of the 3β-axial alcohol (70) into the 3α-equatorial alcohol (71) by dilute alkali followed by esterification with diazomethane, and a cleavage reaction of the 3-ketone (72) to the methyl ester (73) by treatment with sodium hydroxide in methanol at 0° for one hour.



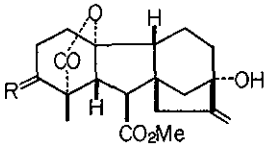
(66)



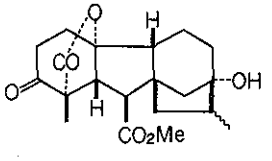
(67)



(68) (69)



(70) R=α-H, β-OH
(71) R=α-OH, β-H



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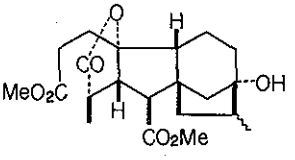


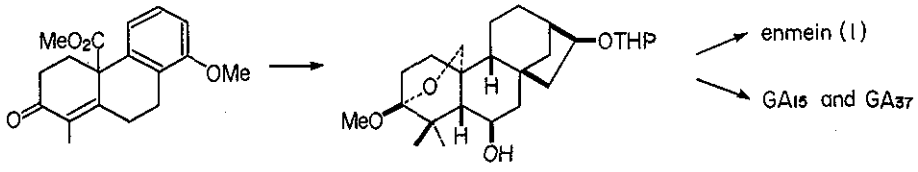
(73)

Okamoto *et al.*[38] carried out the chemical conversion of enmein (1) into *ent*-kaurane (2) independently of us.[1] Subsequently, they succeeded in the chemical conversion of enmein (1) into gibberellin A_{15}.[39] We also accomplished the chemical conversions of enmein (1) into gibberellins A_{15} and A_{37}. *These syntheses of gibberellins are described in section 2-3.*

2-3 Syntheses

As a series of our studies on synthesis of the biologically active diterpenoids, we carried out the synthesis of a tumour inhibitor,[40] enmein (1) and plant hormons, gibberellin A_{15}(GA_{15}) and gibberellin A_{37}(GA_{37}).

ent-16-Kaurene (10) has been recognized as the important precursor of enmein and gibberellins. Enmein (1) is biosynthesised from (10) *via* 6,7-bond fission, and gibberellins are done *via* ring B contraction with extrusion of carbon-7. The stereochemistry at C-5, C-8, C-9, C-10, and C-13 in them is the same as that in *ent*-16-kaurene (10). Hence, it is reasonable to select the norkaurane derivative (75) which can be derived from enmein (1) in good yield as a key intermediate for their synthesis. Actually, we synthesised the racemate of (75) from naphthalene-1,6-diol *via* phenanthrene derivative (74), and accomplished the synthesis of enmein (1) and GA_{15} and GA_{37} from an optically active relay compound (75) derived *from enmein* (1).

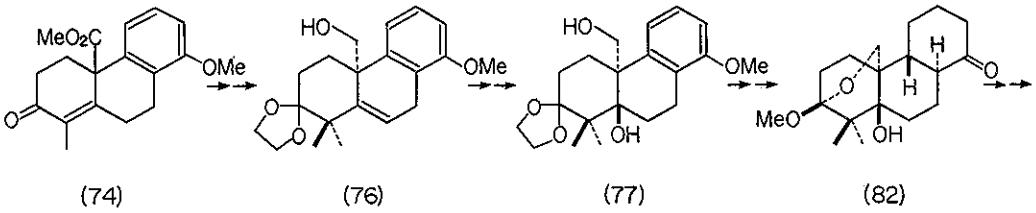


(74) (75)

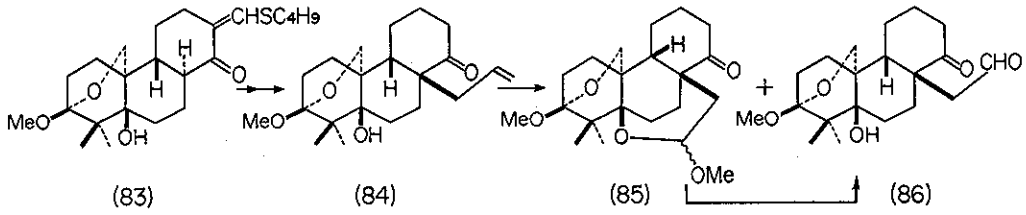
2-3-1 Total synthesis of enmein

2-3-1-1 Synthesis of the compound (75)[41]

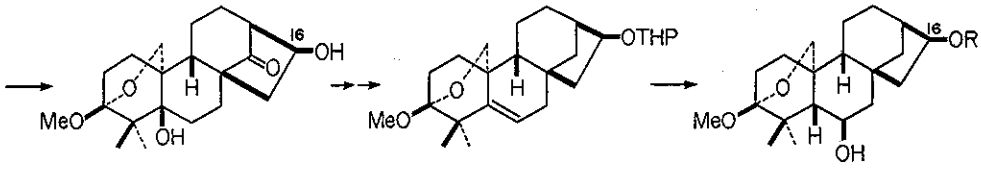
The ketoester (74) synthesised from naphthalene-1,6-diol *via* 5-methoxy-2-tetralone was converted into compound (76) by successive methylation at C-4, ethylene acetalisation at C-3, and reduction of the ester group to the hydroxymethyl group with lithium aluminium hydroxide. Although this compound (76) had the functional groups at the positions which were suitable for the synthesis of (75), it was unstable in air. So this compound was converted to diol (77), which was synthetically equivalent to (76) and stable, *via* epoxide.



(74) (76) (77) (82)



(83) (84) (85) (86)



(87a)
(87b) 16α-ol (88) (75) R=THP
(89) R=H

Scheme 1

Subsequently, the Birch reduction with diol (77) by 18 equivalents of lithium in liquid ammonia followed by acid hydrolysis and subsequent methyl acetalisation gave ketone (82) with *trans*, *transoid*, and *trans* junctures for the rings A, B, and C, respectively, in high yield.

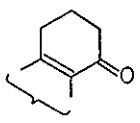
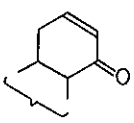
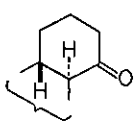
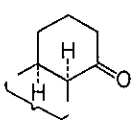
It is noteworthy in this Birch reduction that the product (82) with the desired stereochemistry was obtained in good yield under very mild conditions. In the Table 1, the reactions with (77) are compared with those with (78) and (79).

Table 1 Reaction conditions and products

Entry	Starting material	Li[a] (equiv.)	EtOH (equiv.)	Time (min)	Products[b] (yields %)
1	(77)	10	50	3	I(18),III(34),IV(29)[c]
2	(77)	18	120	40	I(9),III(64),IV(22)
3	(78)	100	300	120	I(10),II(13),III(28)[d]
4	(79)	600	780	120	I(8),II(11)

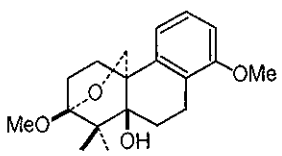
a Concentration (W/V) of lithium in liquid ammonia was 0.3% for entries 1 and 2, 0.7% for entry 3, and ca. 5% for entry 4.

b

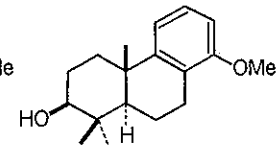
I : 
II : 
III : 
IV : 

c Recovery of the starting material was 9%. *d* Recovery of the starting material was 44%.

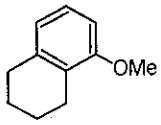
Generally, the Birch reduction with the (80)-type compounds is much more difficult than that with the (81)-type compounds, and only the much less examples have been reported for the former in much less yield than the



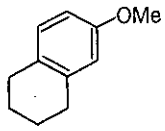
(78)



(79)

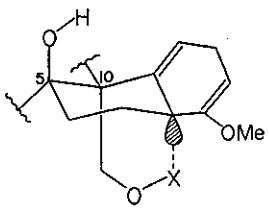


(80)

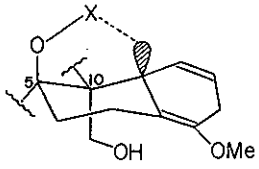


(81)

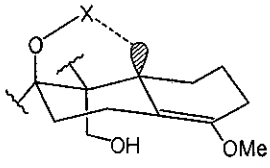
latter. Nevertheless, an extraordinary reactivity of compound (77), a (80)-type compound, was observed, as shown in Table 1. (Compare entry 2 with entry 4.) The reason for this fact is believed to be attributed to the intramolecular participation[42] of the hydroxy groups. The suitable location and stereochemistry of the hydroxy groups appears to control the configuration of the products. The most probable transition states are thought to be A and B for the first step of the reduction and C for the second step.



A



B

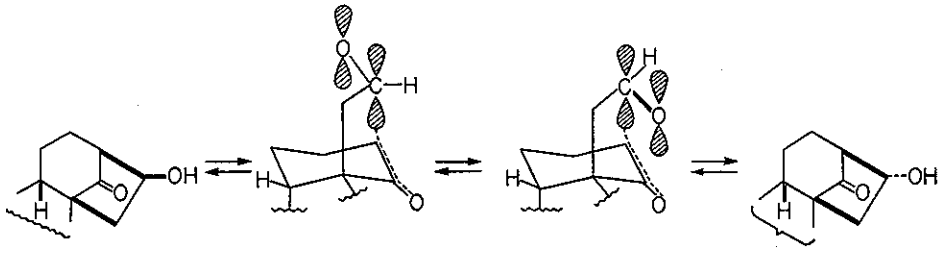


C

The following factor(s) in these transition states may accelerate the reaction and control the stereochemistry of the products : (i) when X=H, stabilisation of the carbanion or transfer of the proton by bridge formation between the hydroxy-proton and the carbanion, and/or (ii) when X=Li, stabilisation of the carbanion by lithium bridge formation between the hydroxy-group and the carbanion, and/or (iii) acceleration of the

formation of the carbanion by electron-transfer through the hydroxy-group.

The next problem is construction of the ring D. The active methylene at C-13 of compound (82) was protected to give (83), which was subjected to allylation at C-8 and subsequent elimination of the protecting group at C-13 by heating with potassium hydroxide in aqueous methanol to yield (84). Ozonolysis of (84) gave a keto-diacetal (85) in addition to aldehyde (86). Thus, it was directly proved that compounds (84) and (85) had the desired *trans, transoid, cis* stereochemistry (Scheme 1). An acidic partial hydrolysis of (85) gave aldehyde (86), which was treated with 0.2% sodium methoxide in methanol for 0.5 hour at room temperature to yield 16β-hydroxy-derivative (87a), the product from a kinetically controlled reaction. The same reaction under more drastic conditions (2.5% sodium methoxide in methanol-tetrahydrofuran at reflux) was thermodynamically controlled to yield the epimeric 16α-ol (87b). These reactions can be tentatively explained by consideration of their transition states, D and E in Scheme 2. The transition state D, where two oxygen functions are far apart, is more stable than E, where they are close and parallel. Hence, the 16β-ol (87a) predominates over the 16α-ol (87b) under kinetically controlled conditions, in spite of unfavoured endosteric interaction in the former.



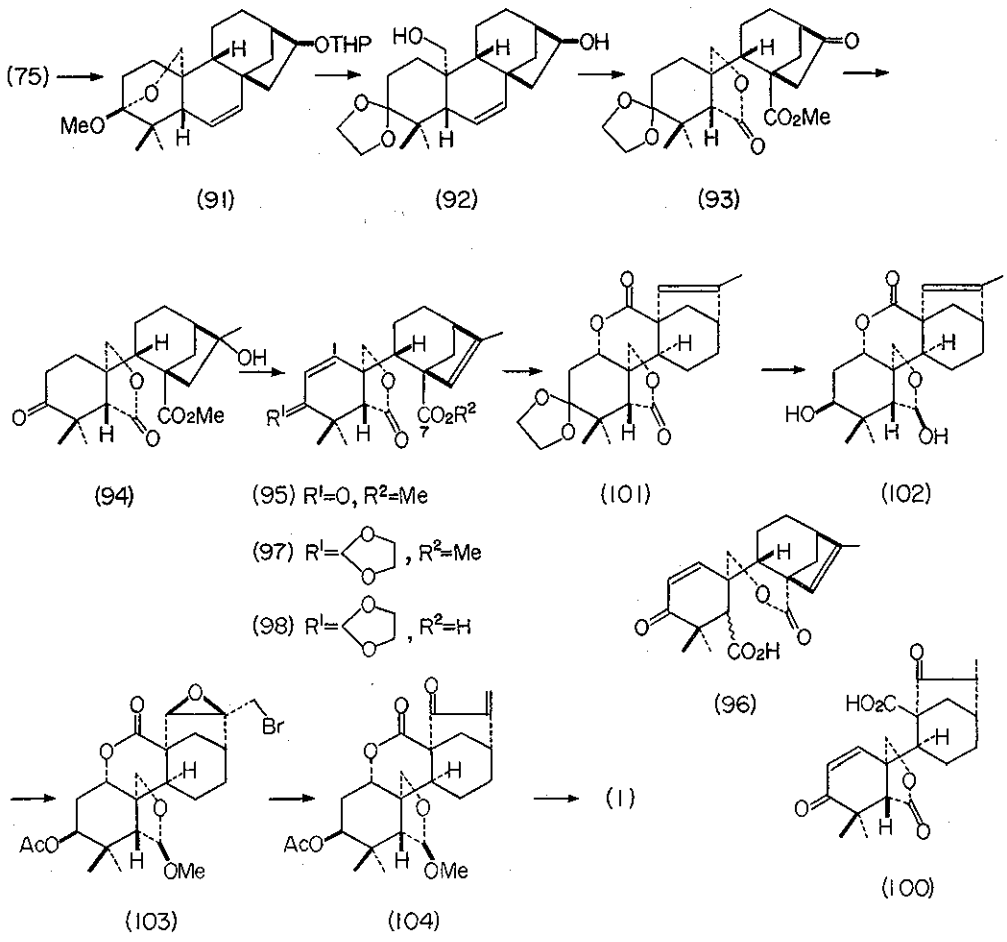
(87a) D E (87b)

Scheme 2

The tetrahydropyranyl derivative of (87a) on Huang-Minlon reduction followed by dehydration with thionyl chloride in pyridine gave 14-deoxo-5-ene derivative (88), which on hydroboration afforded the desired relay compound (75) (Scheme 1). This compound was compared with the optically active compound (75) (Section 2-3-3) derived from enmein (1) and their identity was confirmed. Furthermore, the structure of diol (89), the acid hydrolysis product from (75), was also proved to be correct by comparison with the corresponding optically active diol (89) (Section 2-3-3) derived from enmein (1).

2-3-1-2 Synthesis of enmein from the relay compound (75)[41,43]

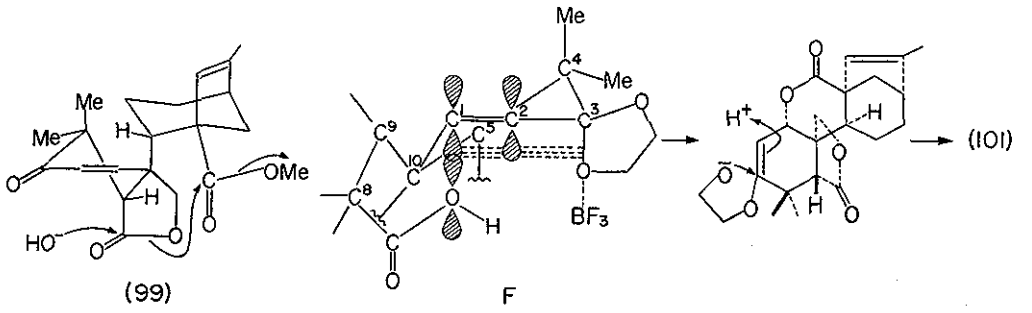
The optically active relay compound (75) derived from enmein (1) through the route described in section 2-3-3, on dehydration with thionyl chloride in pyridine, gave a mixture of isomers, (90)=(88) (rac.) and (91), in a ratio of ca. 1 : 2. The desired isomer (91) was separated from (90) by colum chromatography on silica gel impregnated with silver nitrate, and was subjected to ethyleneacetalisation to give (92), whose ozonolysis product on Jones oxidation followed by methylation with diazomethane yielded the expected 6,7-seco-compound (93). This compound was subjected to the Wittig reaction and treatment with dilute hydrochloric acid to afford the 3-on-16-ol derivative (94). The new hydroxy-group at C-16 was useful to protect the double bond during the next step. Bromination of (94) gave a mixture of two products, which, without purification, was subjected to dehydrobromination by heating in collidine and subsequent dehydration with dimethyl sulphoxide to afford product (95). Purification by recrystallisation was necessary to remove a small quantity of the undersired *exo*-double bond isomer contaminated.



Scheme 3

The next problem is the lactonisation of the carboxylate at C-7 into C-1. Hydrolysis of (95) with weak alkali gave a δ-lactone (96) as the major product, but hydrolysis of acetal (97) did not proceed under such conditions, but under more drastic conditions it gave the desired carboxylic acid (98) quantitatively. The reason why ester (95) is hydrolysed more easily than ester (97) may be due to (i) intramolecular participation of the carbonyl group at C-3, or (ii) predominance of the

concerted reaction shown in formula (99) owing to some influence of the ketone at C-3.



Scheme 4

Previously, lactonisation at C-1 of compound (100) was tried, but the epimeric lactone (having a β C-O bond at C-1) was the major product, while the desired 1α-equatorial lactone was obtained only in 6% yield.[44] On the other hand, a high yield (72%) of the desired lactone (101) was obtained this time by treatment of (98) with boron trifluoride etherate. The reaction produced a sigle product uncontaminated by the C-1 epimer. The high yield on cyclisation is probably attributable to the easy formation of a favoured transition state (F) which satisfies the stereoelectronic requirement for maximum overlapping of the carboxy-group, the π-bond of the double bond, and the C-O bond of the acetal in the α-side of the molecule (Scheme 4).

The compound (101) thus obtained has the same skeleton as enmein (1), and the remaining problems are only reductions at C-3 and C-6 and the modification of the ring D. The ketone obtained by acidic hydrolysis of (101) was subjected to Meerwein-Ponndorf reduction to give 92% yield of the desired 3β-axial hydroxy-compound, a selective reduction of whose γ-lactone was achieved by lithium aluminium hydride in tetrahydrofuran at -30° to

afford hemiacetal (102) (50%). In this way, the first problem was solved. Subsequently, acetalisation at C-6, acetylation at C-3, bromination at the allylic C-17 with N-bromosuccinimide, and epoxidation converted (102) into (103), which on treatment with zinc dust in ethanol at reflux gave an allyl alcohol. Its oxidation with chromium trioxide-pyridine complex afforded an αβ-unsaturated ketone (104). Finally, deacetylation with sodium carbonate solution and hydrolysis with aqueous acetic acid led (104) to enmein (1).

Thus, the total synthesis of enmein (1) *was achieved.* We have reported chemical conversions of enmein into several natural diterpenoids (Section 2-2). Hence this work constitutes formal total syntheses of these natural products.

2-3-2 Total synthesis of gibberellins A_{15} and A_{37}[45,46]

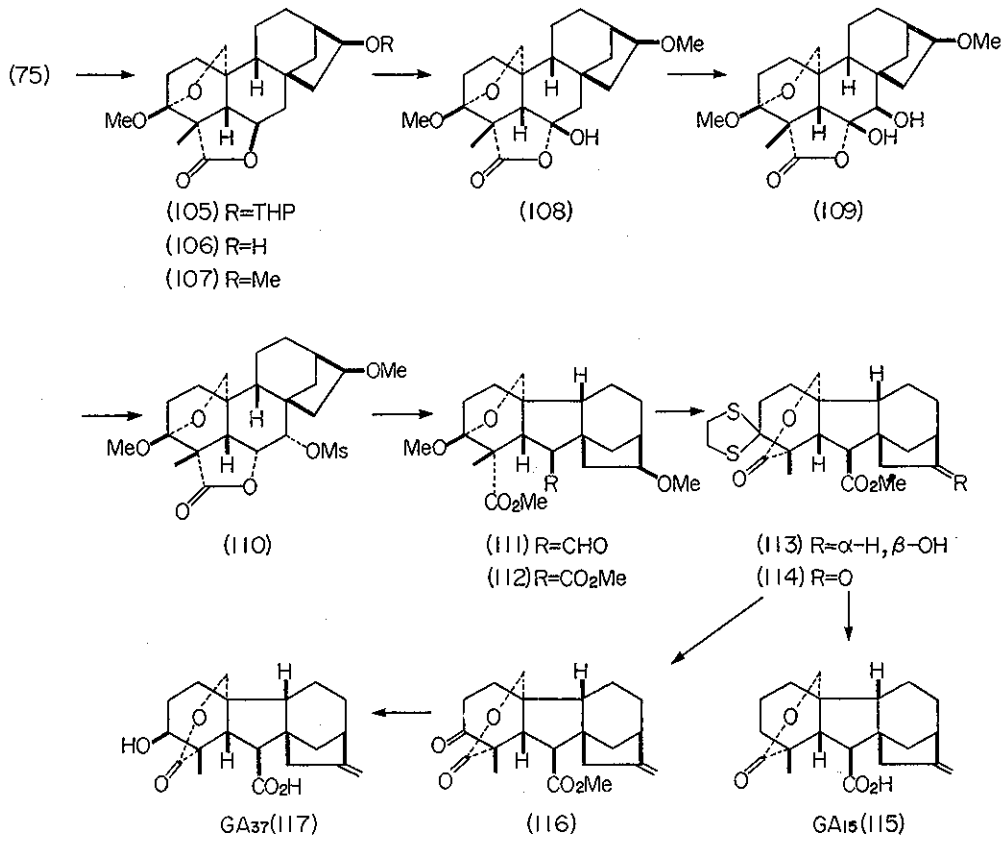
The synthesis of GA_{15} and GA_{37} *from the relay compound* (75) was attempted. There were three important problems in this synthesis. The first one was the introduction of the exocyclic methylene group to C-16. In the total synthesis of enmein (1) it was done at one of the last steps and an alternative substituent which was easily convertible to it was held in the preceding steps, since its introduction in the beginning or middle steps of the synthetic route seemed unfavourable because of its sensitive activity to several reagents. Also in this synthesis it was introduced at a step near the end. *Before its introduction, the 16β-hydroxy group had to* be protected by some suitable group. Since we found an excellent demethylation agent,[47] the methyl group which was very stable to general regents was effectively used for protection of the 16-ol. The second problem was oxidation of the C-19 methyl group. On the basis of the preliminary experiments of the hypoiodite reaction with various kaurane-6-

ol derivatives, the compounds in which the ring A was fixed as the boat form and the 6-ol had an equatorial conformation, for instance (75) and (126), were found to be the most suitable materials for the oxidation of the C-19 methyl group.[48] The third problem was the contraction of the ring B. As the result of the preliminary experiments on several kaurane type derivatives, compound (110) was found to give rise to the gibberellane derivative quantitatively.[49] From the foregoing facts, a route from (75) to GA_{15} and GA_{37} *via* (110) was build (Scheme 5).

The relay compound (75) was subjected to the hypoiodite reaction to give 19,6β-olide (105), whose treatments with diluted hydrochloric acid and subsequently with diazomethane and boron trifluoride etherate afforded 16β-methoxy compound (107) *via* 16β-ol (106). The hydrolysis and subsequent Jones oxidation converted the lactone (107) into 6β-hydroxy-19,6α-olide (108), whose dehydration, epoxidation, and treatment with acid gave 6β,7β-diol derivative (109). Lithium aluminium hydride reduction of (109), Jones oxidation, sodium borohydride reduction, and mesylation led to conversion of (109) into (110) in good yield. Treatment of (110) with potassium hydroxide in t-butanol and esterification (diazomethane) yielded the desired gibberellane aldehyde (111) quantitatively. On Jones oxidation and methylation (111) gave ester (112).

The remaining problems in its transformation into gibberellins are the modifications of the rings A and D. By using the boron trifluoride-thiol system which we found and developed, the compound (112) was directly transformed into (113) under the simultaneous occurrence of dithioacetalisation at C-3, lactonisation between C-20 and C-19, and demethylation at the C-16 methoxy group. The 16-ol (113) on Jones

oxidation afforded the ketone (114), an important common intermediate for synthesis of C_{20} gibberellins (*e.q.* GA_{15}, GA_{27}, and GA_{37}).



Scheme 5

The Raney nickel catalysed reductive desulphurisation of (114) and subsequent Wittig reaction yielded gibberellin A_{15} methyl ester, which on treatment with lithium propylmercarptide in hexamethylphosphortriamide (HMPA)[50] afforded the desired GA_{15} (115). Thus the total synthesis of GA_{15} was accomplished. Since the first total synthesis of GA_{15} has been

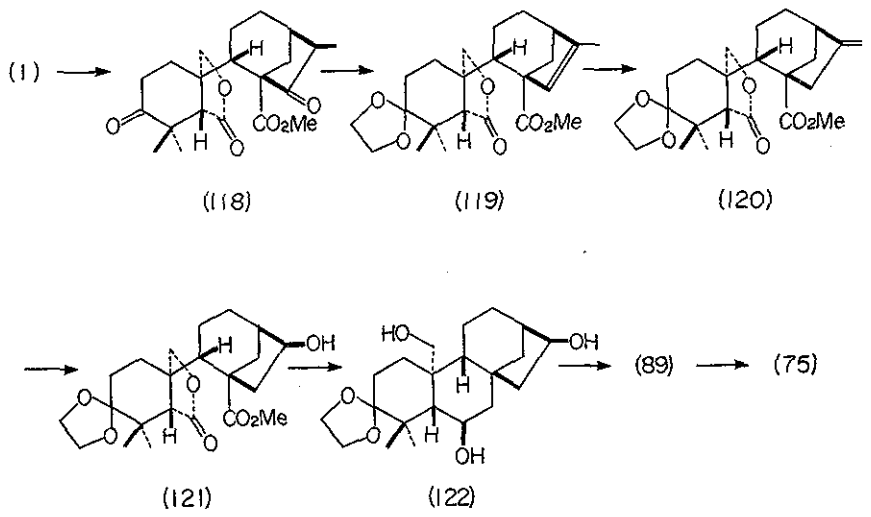
done by Nagata *et al.*[51] in 1970, this work means the second synthesis.

The wittig reaction and dethioacetalisation by N-chlorsuccinimide[52] converted (114) into (116). The 3-ketone was subjected to the Meerwein-Ponndorf reduction to give the 3β-ol, that is, GA_{37} methyl ester as the major product. The demethylation of the ester was effected by the foregoing procedure[50] to yield GA_{37} (117). Thus the first total synthesis of GA_{37} was accomplished. The chemical conversion of (114) into GA_{27} is in progress. The synthesised optically active GA_{15} and GA_{37} were proved to show the physiological activity as expected, which will be published elsewhere.

2-3-3 The chemical transformation of enmein into the relay compound (75)[53]

The diketo-lactone ester (118)[1] derived from enmein (1) on partial acetalisation, sodium borohydride reduction in aqueous methanol, mesylation and heating in dimethyl sulphoxide afforded the 15-ene (119) (Scheme 6). Allylic bromination of (119) followed by zinc-duct reduction gave the desired exocyclic methylene compound (120). Ozonolysis of this compound and subsequent sodium borohydride reduction gave 16β-ol (121), which on treatment with sodium in liquid ammonia afforded triol (122) as the major product. The compound was refluxed in a mixture of methanol and chloroform in the presence of conc. sulphuric acid to yield a methyl acetal diol (89), which on partial tetrahydropyranylation yielded the desired compound (75).

Since the compound (75) has been converted into GA_{15} and GA_{37} (2-3-2), this constitutes the chemical conversion of enmein (1) into these gibberellins.

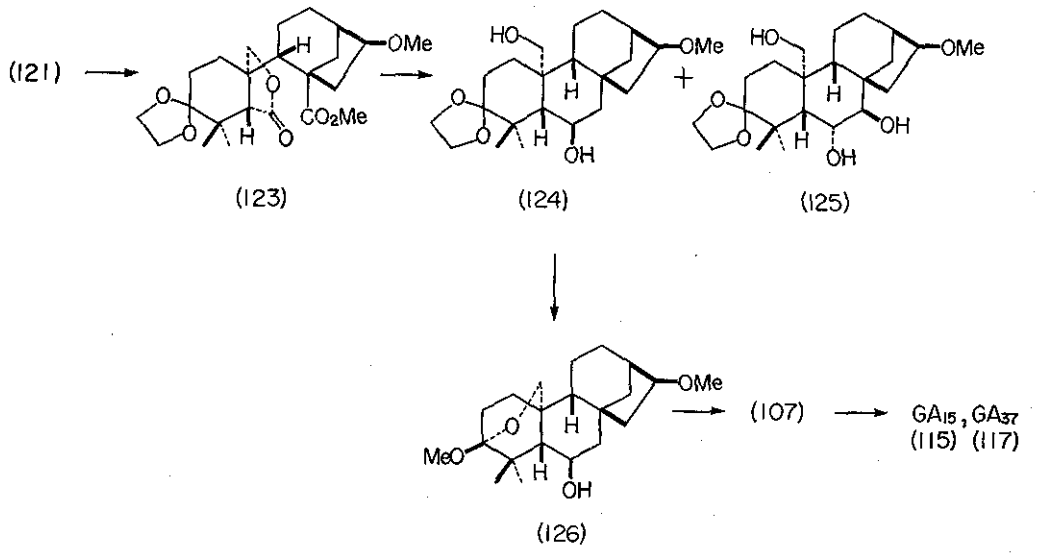


Scheme 6

2-3-4 Synthesis of gibberellins A_{15} and A_{37} from enmein[45,46]

Although a synthesis of GA_{15} (115) and GA_{37} (117) from enmein (1) has been accomplished as stated in the preceding section (2-3-3), the overall yield from (121) to (105) *via* (75) was poor. Hence, an improved modification for the route was successfully tried.

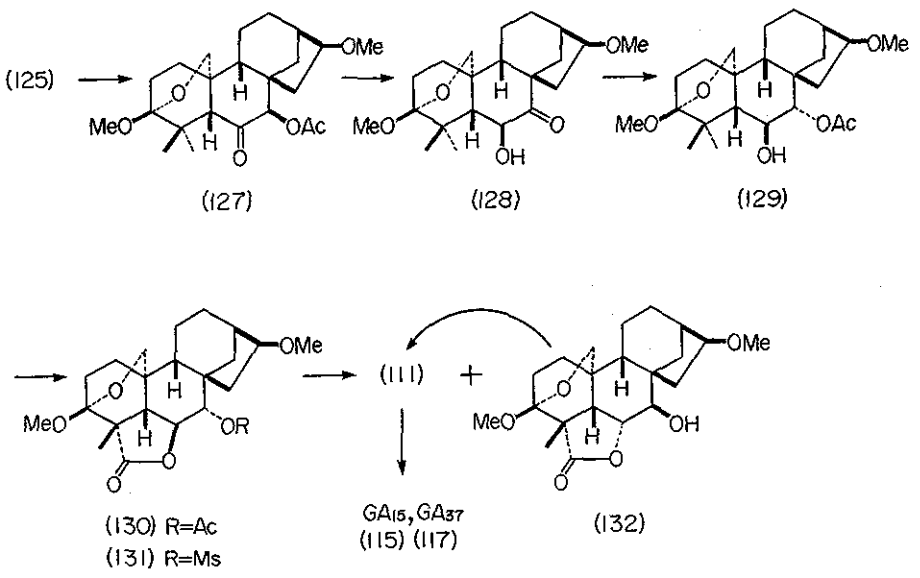
The 16-ol (121) was transformed to methyl ether (123) by methyl iodide and sodium hydride, and it was treated with sodium in liquid ammonia. In this reaction, the use of 25-30 atom equivalents of sodium gave the best yields of diol (124) (ca. 50%) and triol (125) (25-35%). The diol (124) was transacetalised to (126) by methanol in the presence of sulphuric acid and it was subjected to the hypoiodite reaction to give lactone (107) in good yield (Scheme 7). The compound (107) was incorporated into the synthetic route of gibberellins as described in Section 2-3-2.



Scheme 7

The triol (125) was converted into (129), a suitable material for the hypoiodite reaction, as follows. The transacetalisation at C-3, acetylation at C-7, and Jones oxidation of 6-ol converted (125) into acyloin acetate (127), which on treatment with alkali gave the desired acyloin (128) possessing 6β-ol almost quantitatively. On the Meerwein-Ponndorf reduction, the acetate of (128) gave 6β,7α-diol, which was acetylated to give the desired 7α-acetoxy-6β-ol (129) (Scheme 8).

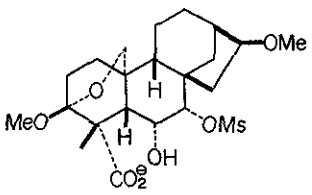
The compound (129) on the hypoiodite reaction gave lactone (130) as the major product, which on partial hydrolysis (sodium carbonate) and mesylation gave 7α-mesylate (131). The base catalysed rearrangement with (131) and esterification of the product with diazomethane gave the desired gibberellane aldehyde (111) and a by-product (132) in a ratio of ca. 1 : 1. Both products were converted into GA_{15} and GA_{37} as described in Section 2-3-2.



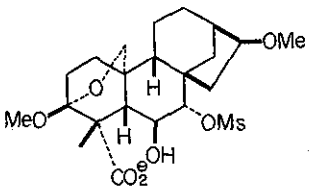
Scheme 8

It is noteworthyl that the ring B contraction with (110) proceeded very smooth to yield (111) quantitatively, while (131) gave only 50% yield of (111) in the same reaction. In the compound (110), the ring B has the twisted boat form, hence the mesyloxy group at C-7 takes a quasi axial conformation, which is apparently unfavourable for the ring B contraction. On the other hand, the ring B in the compound (131) has a chair conformation, hence the mesyloxy group at C-7 takes an equatorial conformation, which is apparently favourable for the ring B contraction because of the anti coplanar relation between the 5,6 carbon-carbon bond the 7-oxygen bond. The foregoing results are reasonably explained by the first opening of the lactone ring and subsequent ring contraction *via* a favourable transition state, that is, an anti coplanar conformation between the migrating 5,6 carbon-carbon bond and the 7-oxygen bond in the leaving group. Thus, the former (110) forms (133), whose ring B changes from the original twisted boat form to a chair form for the release of the non-

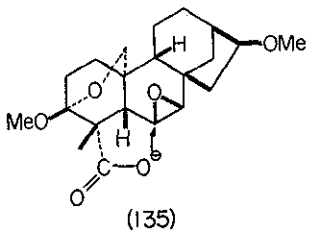
bonded interaction between the 19-carboxylate and the 6α-hydroxy group, so that a favourable anti coplanar conformation is build between the migrating bond and leaving group. On the other hand, the latter (131) forms (134), whose ring B tends to change to a boat form for the release of the same non-bonded interaction. From the boat form of the ring B, the epoxide (135) is formed and the attack of the carboxylate from the α-side causes the epoxide ring opening to yield (132).[49]



(133)



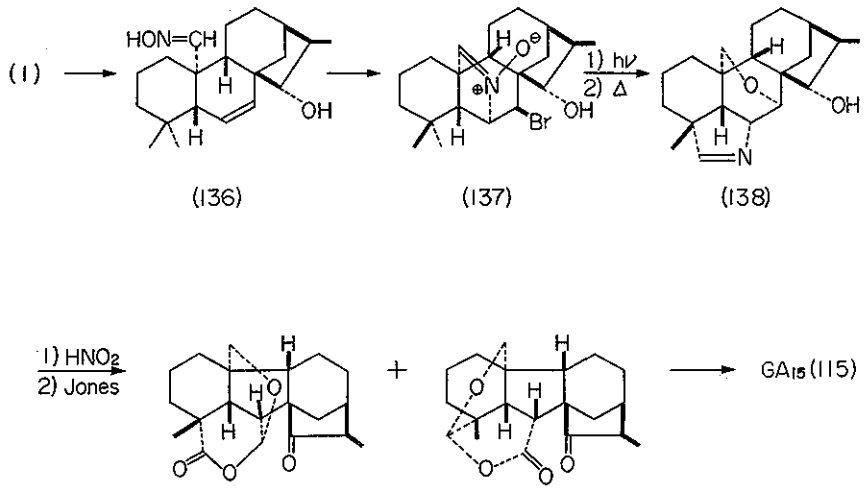
(134)



(135)

Thus, diol (124) and triol (125) prepared from (123) by the modified acyloin condensation were transformed into gibberellane derivative (111) in good yields, and they were effectively supplied for the total synthesis of GA_{15} and GA_{37}.

Independently, Okamoto *et al.*[39] preformed the first chemical conversion of enmein (1) into GA_{15} (115). This synthesis is characteristic of oxidation at C-19 by photolysis of the nitrone (137) derived from enmein (1) *via* (136) and of the ring B contraction of (138) using nitrogen atom at C-6 (Scheme 9).



Scheme 9

2-4 Biosynthesis

Enmein (1) and oridonin (8) have been thought to be biosynthesised through the pathway similar to that of general cyclic diterpenes, and *ent*-16-kaurene (10) has been regarded as an important precursor for them. Thus oridonin (8) may be formed *via* oxygenations at C-1, -6, -7, -14, -15, and -20 of *ent*-16-kaurene (10), and enmein (1) is assumed to be formed *via* oxidative cleavage of the 6,7 carbon-carbon bond of an oridonin-like precursor.[44]

All of the thirty kinds of diterpenoids isolated from *Isodon* species have the oxygen functions at the C-7 and C-15 positions. Hence these carbon atoms were assumed to be oxidised at one of the early stages in the biosynthetic course from *ent*-16-kaurene (10). On the basis of these considerations, a biogenetic pathway was proposed.[27]

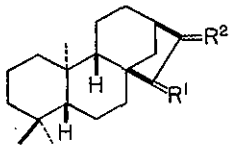
Functionalisation at C-15 may occur through one of the following

pathways : (i) a direct oxygenation at the allylic position of *ent*-16-kaurene (10), (ii) oxygenation of *ent*-15-kaurene (50) by a singlet oxygen accompanied by allylic rearrangement (ene reaction), or (iii) epoxidation of (50) followed by rearrangement. As the results of the feeding experiments of the isotope labelled compounds to *I. japonicus* plants, *ent*-16-kaurene (10) and *ent*-16-kauren-15-one (139) were proved to be incorporated into enmein (1) and oridonin (8), and the pathway (i) was supported for the functionalisation at C-15. In this section these investigations are described.

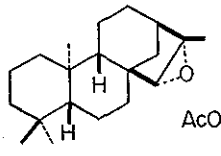
As a preliminary experiment of the biosynthesis, changes in the quantity of major diterpenoids during growth of *I. trichocarpus* were examined by gas chromatography and combined gas chromatography-mass spectrometry.[54] A change in the quantity was found by plotting the area ratio of trimethylsilylated diterpenoids to that of internal standard in gas chromatography every ten days. Enmein (1) and oridonin (8) showed a similar tendency of changes in their quantity. They were found to increase markedly in June and July.

Now, the C-17 labelled following compounds were prepared[57,58,59] : *ent*-15-kaurene (50), *ent*-16-kauren (10), *ent*-15β,16-epoxy-kaurane (140), *ent*-16-kauren-15β-ol (141), *ent*-16-kauren-15-one (139), *ent*-16-kauren-15α-ol (142), and *ent*-kauran-15-one (143). Three methods of feeding, *i.e.*, infiltration, hydroponics, and application, were examined using [17-^{14}C]-*ent*-16-kaurene. As the result, application proved to be most suitable. Thus, the labelled compounds, dissolved in acetone, respectively, were applied to the reverse side of leaves of the growing *I. japonicus* plants, and the leaves were harvested after a week. Enmein (1) and oridonin (8) were isolated and purified as diacetate (144) and tetraacetate (145),

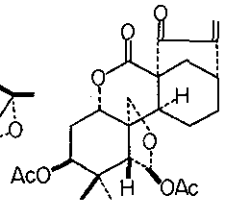
respectively. Consequently, *ent*-16-kaurene (10)[57,59] and *ent*-16-kauren-15-one (139)[58,59] proved to be incorporated into both enmein and oridonin. *ent*-15-Kaurene (50), 15,16-epoxide (140), alcohol (141), and *ent*-kauran-15-one (143) were not incorporated into both diterpenes. *ent*-16-Kauren-15α-ol (142) was incorporated into enmein, but not into oridonin. These observations deny the pathways (ii) and (iii) for the functionalisation at C-15 of *ent*-kaurene, but support the pathway (i), *i.e.*, a direct oxygenation at the allylic 15-carbon atom of *ent*-16-kaurene (10). The 15-ketone may be formed *via* hydroperoxidation from the less hindered α-side of the ring D of *ent*-16-kaurene (10) followed by dehydration.



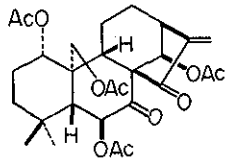
(139) R^1=O, R^2=CH_2
(141) R^1=α-OH, β-H, R^2=CH_2
(142) R^1=α-H, β-OH, R^2=CH_2
(143) R^1=O, R^2=α-H, β-Me



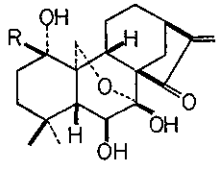
(140)



(144)



(145)



(146) R=^{3}H
(147) R=H

Ozonolysis of the radioactive enmein diacetate (144), in which C-17 labelled (10) or (139) had been incorporated, led to nearly quantitative recovery of the radioactivity from C-17 as formaldehyde, demonstrating specific incorporation in both cases. In contrast, the recovery of radioactivity from (144), in which C-17 labelled (142) had been incorporated, was 58.1%. Generally, separation of pure dihydroenmein and

enmein from the mixture is very difficult. *Isodon japonicus* plants have been shown to contain enmein uncontaminated by dihydroenmein.[2] The n.m.r. spectrum of the radioactive enmein diacetate obtained from the plant to which (142) had been fed gave no evidence for contamination by dihydroenmein diacetate, but the low localisation of the radioactivity at C-17 was observed in this case. We suggested the following explanation for this. (i) The 15β-ol (142) was enzymatically or non-enzymatically converted to *ent*-kauran-15-one (143) by a garryfoline-cuauchichicine type rearrangement;[56] and then into dihydroenmein. (ii) The content of dihydroenmein was so low that it was not detected by the n.m.r. spectroscopy. (iii) Dihydroenmein has a higher specific radioactivity than enmein. But, a further detailed investigation will be required.

Thus, it was clarified that *ent*-16-kaurene (10) is an important precursor to the diterpenes of *I. japonicus*, and a triplet oxygen is related to oxygenation at the allylic 15-position of (10).

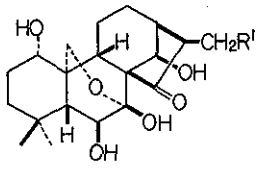
In addition, incorporation of [1β-^{3}H]-14-deoxyoridonin (146) into oridonin (8) by *I. japonicus* was also demonstrated by tracer experiments.[58]

Now, the tracer experiments with the radioactive kaurene derivatives having an oxygen function at C-7, -6, or -20 as well as having two oxygen functions, for instance, at C-7 and C-15, have been carried out or are being attempted.[60]

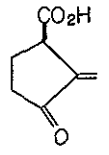
2-5 Biological activity[40,61]

Isodon japonicus Hara and *I. trichocarpus* Kudo have been used as the home remedy in Japan, but their essential phisiological or biological activity has not yet been clarified. As described in Section 2-1, many diterpenoids which contained an α-methylene cyclopentanone system in their

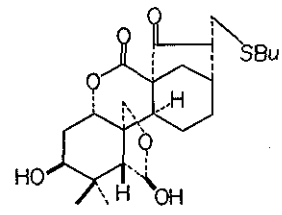
molecule have been isolated from these plants. We expected that this conjugated system would contribute to the antitumour activity, if the diterpenoids are active. Then, the antitumour activity of some available diterpenoids and their derivatives against Ehrlich ascites carcinoma was investigated by their i.p. injection of 5∿40 mg/Kg every 24 hours after tumour inoculation to mice for 7 days, followed by observation for 33 days. As the result, oridonin (8) and lasiokaurin (28) showed a significant activity as expected. Enmein (1), enmein 3-acetate (3), compound (58)[7b] derived from oridonin (8), and compound (147)[33] derived from trichokaurin (4) were also shown to be active, under a higher dose than that of oridonin (8). Oridonin dihydro-derivative (148),[7b] oridonin butane thiol adduct (149),[61] dihydroenmein, and trichokaurin (4), however, did not show any activity. From these facts, the α-methylene cyclopentanone function was established to be the important active centre for the activity. The antitumour natural products having an α-methylene cyclopentanone system had not been known, except for sarkomycin (150).[62,63]



(148) R^1=H
(149) R^1=S$(CH_2)_3$Me
(151) R^1=SR^2;
R^2=Et, Pr, sec.Bu,
or $CH_2CH(NH_2)CO_2H$



(150)



(152)

The relationship between activity and structure was analysed. The antitumour activity of oridonin (8) and lasiokaurin (28) was estimated to come from satisfying the following necessary conditions : (i) As an active centre, an α-methylene cyclopentanone is present in the molecule. (ii)

Some hydroxy group(s) is present at the position suitable for contact with and binding with an enzyme containing a specific nucleophile. (The β-hydroxy group at the 14 position and/or the hydroxy group at the 7 position.) (iii) A hydrogen bonding between the hydroxy group at the 6 position and the carboxyl group at the 15 position is present to enhance the electrophilicity of the carbon atom at the 17 position.

Subsequently, the antibacterial test of oridonin (8), lasiokaurin (28), compound (147), enmein (1), enmein 3-acetate (3), compounds (148) and (149), trichokaurin (4), and dihydroenmein was carried out. Compounds (8), (28), (147), (1), and (3) showed activity against gram-positive bacteria, while compounds (148), (149), (4), and dihydroenmein did not indicate any activity. The activity of oridonin (8), lasiokaurin (28), and compound (147) was higher than that of enmein (1) and enmein 3-acetate (3). Thus the specific activity against the gram-positive bacteria was observed in the compounds which had an α-methylene cyclopentanone system in their molecule. Furthermore, similarly to the antitumour activity, the hydrogen bonding between the 6-hydroxy and 15-carbonyl groups in oridonin (8) played an important role for increasing the antibacterial activity.

The mechanism for the appearance of the activities was assumed by the following biomimetic reactions with oridonin (8) and enmein (1). Adenosine and cytidine, as the nucleic acid model compounds, and four kinds of alkane thiols, L-cysteine, L-lysine, and L-serine, as the enzyme model compounds, were allowed to react with the diterpenoids. As the result, oridonin (8) did not cause any reaction with adenosine and cytidine in a buffer solution, but was recovered. The reactions of oridonin (8) with the SH enzyme model compounds easily proceeded stereoselectively under mild conditions to give alkane thiol adducts (149) and (151). Enmein (1) also easily gave adduct

(152) by the reaction with butane thiol.

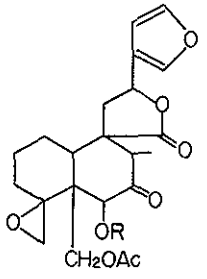
In conclusion, our biomimetic reactions supported the biologically active centre of *Isodon* diterpenoids to be α-methylene cyclopentanone function and the hypothesis that the appearance of the biological activity may be due to the *in vivo* deactivation of the SH enzyme by these diterpenoids.

Finally, the papers on the biological activity of the *Isodon* diterpenoids by other groups are briefly shown. Arai, *et al.*[64] reported that the crude crystalline substance obtained from *I. japonicus* and *I. trichocarpus* indicated an antitumour activity. Subsequently it was shown by the same group[65] that purified enmein and its acetate indicated an antitumour activity, while dihydroenmein indicated no activity, although only the partial structure had been proposed for enmein in that time. Kubota *et al.*[66] reported the relationship between the structure and the bitter taste of the *Isodon* diterpenoids. They also reported recently the antibacterial activity of the *Isodon* diterpenoids.[67]

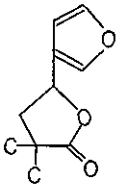
3 Diterpenoids of *Teucrium* plants

The investigation on diterpenoids of Labiatae in our laboratory was extended from the *Isodon* to the *Teucrium* genus. Thus a new crystalline norditerpene, teucvin ($C_{19}H_{20}O_5$) was isolated from the neutral extract of *Teucrium viscidum* Blume *var. Miquelianum* (Maxim.) Hara.[68] Teucvin was shown to contain two lactone groups giving pKa' values of 8.4 and 6.5 by lactone titration. Its i.r. absorptions at 1600, 1505, and 875 cm^{-1} and positive Ehrlich test indicated the presence of a furan ring. Thus, all the oxygen functions were characterized. Previously pikropolin was isolated from *Teucrium polium* by Brieskorn and Pfeuffer and it was assigned structure (153).[69] By the comparison of its n.m.r. data with those of

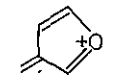
teucvin, the presence of such a partial structure (154) in the teucvin molecule as in pikropolin was suggested, which was supported by mass spectral fragmentations into (155) and (156).



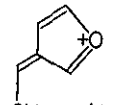
(153) R=H
(163) R=Ac



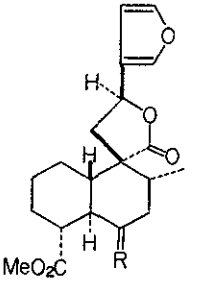
(154)



(155)

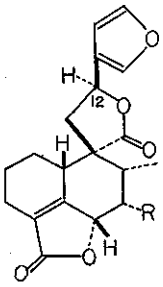


(156)

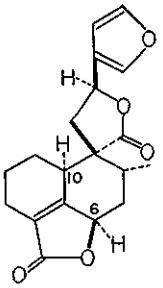


(157) R=α-OCOCH$_2$Br, β-H
(158) R=α-OH, β-H
(159) R=O

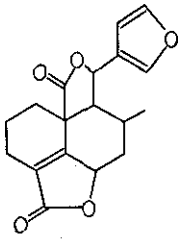
Teucvin was treated with sodium carbonate in refluxing methanol to give a crystalline product, $C_{20}H_{24}O_6$, in good yield. This compound was presumed to be a keto-ester on the basis of i.r. and n.m.r. data. Its reduction with sodium borohydride afforded a hydroxy-ester. The bromoacetate of this hydroxy-ester was prepared as orthorhombic single crystals, which were shown to have structure (157) including the absolute configuration by X-ray analysis. Hydrolysis of (157) with sodium carbonate in refluxing methanol gave the hydroxy-ester, whose Jones oxidation regenerated the foregoing keto-ester. Thus the structures (158) and (159) were assigned to the hydroxy-ester and keto-ester, respectively. The structure of teucvin itself was established as (160) on the basis of the results of several reactions, *i.e.*, catalytic hydrogenation, oxidation with osmium tetroxide, Birch reduction, treatment with sodium carbonate in refluxing deuteriomethanol *etc.*[68,70]



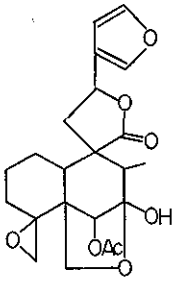
(160) R=H
(165) R=OH



(161)



(162)



(164)

Subsequently, a minor component, teucvidin was isolated from the same plant. Its structure (161) was assigned except for the stereochemistry of C-12 on the basis of spectroscopic data, especially n.m.r. and INDOR spectroscopic investigations.[71] The X-ray analysis of teucvidin provided an evidence for the structure and relative stereochemistry (161).[72] Finally, comparison of the c.d. spectrum of teucvin with that of teucvidin and investigation of the conformation of each ring in both compounds by n.m.r. and INDOR spectroscopy led to establishment of the absolute configuration (161) for teucvidin. Thus teucvidin was proved to be a diastereoisomer of teucvin (160) at C-6 and C-10.

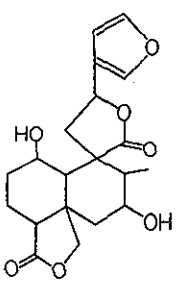
In relation to these studies, teucvin (160) was isolated from *Mallotus rependus*, a plant of Euphorbiaceae collected in Borneo, by Itô *et al.* They recognized again its structure (160) by an X-ray analysis of an exocyclic adduct of *p*-bromophenylmaleinimide to teucvin.[73] Dominguez *et al.* in Mexico isolated a norditerpene, "eugarzasadone", from *Teucrium cubense*, and proposed structure (162).[74] But later this compound was proved to be identical with teucvin,[75] and the structure was revised to (160).[76]

As described above, Brieskorn and Pfeuffer[69] isolated pikropolin from

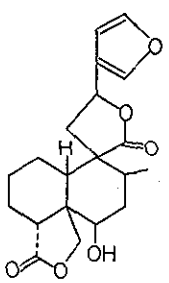
T. polium and determined its structure as (153). They also isolated other two related compounds (163) and (164),and elucidated their structures as shown.

In Soviet Russia, Reinbol'd *et al.* isolated four bitter diterpenes from *T. chamaedrys* and characterized their functional groups.[77] To teucrin A, one of them, was assigned structure (165) except for the configuration of C-12.[78,79] Subsequently its absolute structure (165) was established on the basis of the spectral and chemical evidence.[80] Furthermore, the structures of teucrins B, E, F, and G isolated from the same plant were proposed as (166), (167), (168), and (169), respectively, on the basis of the i.r., c.d., and n.m.r. spectral data and some chemical reactions.[81]

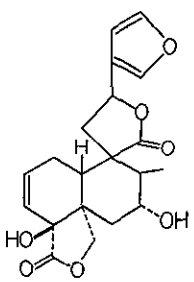
Chatterjee in India recently isolated a diterpene from the petrol-extract of the stem-bark of *Croton caudatus* Geisel (Euphorbiaceae).[82] It was proved to be identical with teucvidin (161) by its direct comparison with the authentic sample.



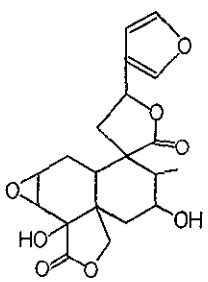
(166)



(167)



(168)



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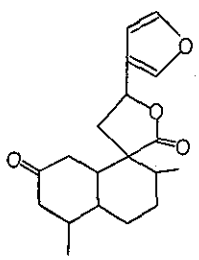

(170)

The *Teucrium* diterpenes found so far have been shown to have a common 19-norclerodane or clerodane skeleton which contained a spiro γ-lactone and a β-substituted furan ring. It is interesting that Chan *et al.*[83] had isolated crotonin (170), whose structure corresponded to simpler and

principal skeleton of the *Teucrium* diterpenes, from *Croton lucidus*.

Finally, it seems significant to describe that teucvin (160) and teucvidin (161) indicate anti bacterial activity against some gram-positive bacteria.[84] It was also reported that eugarzasadone (teucvin) showed *in vitro* very potent amoebicide activity.[74]

4 Conclusion

As described above, we have carried out chemical investigations on the diterpenoids of *Isodon* and *Teucrium* plants. Many kinds of diterpenoids were isolated and their structures elucidated. Chemical conversions with each other *via* biogenetic or retro-biogenetic route were done. Total synthesis of enmein, GA_{15} and GA_{37} have been accomplished. Some biologically active diterpenes were found. The feeding experiments began for elucidation of the biosynthetic pathway. Thus the sound basis in the field of the chemistry on the *Isodon* and *Teucrium* diterpenoids has been established.

Now, several new attempts are in progress. Especially, we are developing the synthetic research of the biologically active related diterpenoids, for instance, oridonin and other gibberellins. The biosynthetic research will be developed systematically. Furthermore, we are going to develop new biologically active compounds on the basis of the results of our research works obtained so far.

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