Contribution

Syntheses of Catechol Diterpenes and Their Biological Activities

Masahiro TADA

Professor Emeritus
Tokyo University of Agriculture and Technology
Fuchu, Tokyo 183-8509, Japan

1. Biological activities of catechol diterpenes

A bioactive organic compound has a unique three-dimensional structure with specific functional groups in required positions on the skeleton. In general, derivatives of the same skeleton without the functional groups show very low or no biological activity. Synthesis of a bioactive compound requires easy introduction of the functional groups into the skeleton. Successful synthesis of a bioactive compound with a complex structure may result in the discovery of novel organic reactions, but may provide only a few mg of the desired compound. However, investigation on the biological activities of a compound requires a large amount of the compound. Development of a facile synthesis of an important bioactive compound in large quantities may produce new science.

Recently, many studies on the bioactivity of anti-oxidants in food have been reported. Anti-oxidants in foods include vitamins, sulfur compounds, and many polyphenols. Because the polyphenols can be oxidized by oxygen, they are unstable in air. Natural polyphenols can be classified as flavonoids (*e.g.*, catechins in teas), stilbenes (*e.g.*, resveratrol in wines), phenolic diterpenes (anti-oxidants in rosemary), phloroglucinols (anti-oxidants in hops) and so on. Many anti-oxidants also show other various bioactivities, such as plant defense compounds against fungi, bacteria, or insects.[1-10]

2. *Ortho*-quinone and *ortho*-quinone methide

Among the polyphenols, catechols are easily oxidized to *ortho*-quinones, which are tautomeric isomers of 2-hydroxy-1,4-

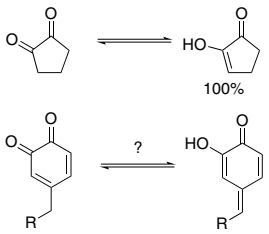

Fig. 1. Tautomeric isomerizations between cyclopenta-1,2-dione and 2-hydroxy-2-cyclopentenone, and between *ortho*-quinone and *ortho*-quinone methide.

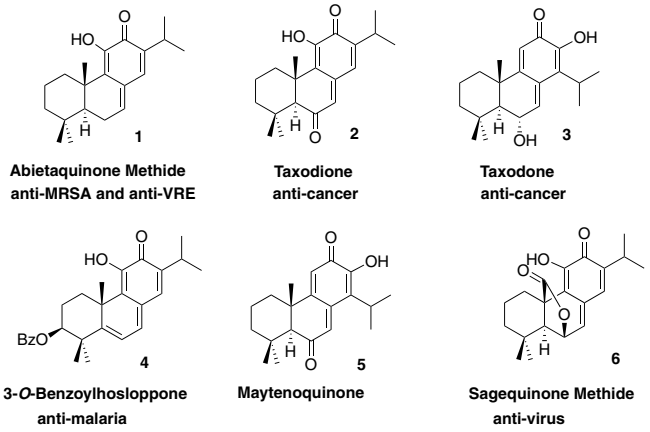

Fig. 2. Diterpenes with the quinone methide structure.

quinone methide (*ortho*-quinone methide). As both tautomeric isomers are unstable, few reports on the chemical reactivity and bioactivity of *ortho*-quinone methides exist.

No study on the equilibrium bias between *ortho*-quinone and *ortho*-quinone methide has been reported. The tautomeric equilibrium between *ortho*-quinone and *ortho*-quinone methide is similar to the tautomeric equilibrium between cyclopenta-1,2-dione and 2-hydroxy-2-cyclopentenone. In the equilibrium, 2-hydroxy-2-cyclopentenone exists as the only isomer (Fig. 1).

The MOPAC calculation of abietaquinone methide (**1**), a natural diterpene, showed that the major isomer is *ortho*-quinone methide, which is in agreement with our experiment.[6,11] Many stable natural *ortho*-quinone methide diterpenes have been reported, *e.g.*, abietaquinone methide[11] (**1**) (possesses anti-MRSA and anti-VRE activity, and is used as a remedy for intestinal worms in east Africa), taxodione[12-14] (**2**) and taxodone[12-14] (**3**) (possess anticancer activity), 3-*O*-benzoylhosloppone[10] (**4**) (possesses antimalarial activity), maytenoquinone[13,16-18] (**5**) and sagequinone methide[3] (**6**) (possess anti-viral activity) (Fig. 2). Therefore, we investigated the synthesis and the bioactivities of diterpenes with quinone methide and related compounds.

3. Total syntheses of diterpenes with an abietane skeleton *via* the stereo-selective cyclization of polyenes

Diterpenes with an abietane skeleton are biologically synthesized *via* sequential cyclizations of geranylgeranylpyrophosphate. For our total synthesis of abietane diterpenes, the stereo-selective cyclization of a modified polyene ester (**7**) was examined.[19]

The modified polyene **7** produced an asymmetric center at C7 in the cyclized products (**8** and **9**). Stereochemistry of the products was changed due to the alkyl group of the ester at C7 during the cyclization of **7** with Lewis acid $BF_3 \cdot OEt_2$ in nitromethane (Fig. 3). Larger alkyl esters such as isopropyl or menthyl produced more stereoselectivity compared to the methyl ester (Fig. 3). This selectivity can be explained by the difference in stability between two transition states (**I** and **II**) to give **8** and **9**, respectively. Transition state **I** may have greater steric repulsion between the ester group and the aromatic hydrogen, compared to transition state **II**. Therefore, the larger alkyl ester **7** afforded **9** selectively through the more stable transition state **II** during cyclization.

This stereoselective cyclization of modified polyenes was applied to the total syntheses of 12 diterpenes. The synthesis of racemic ferruginol[20,21] (**16**) from the cyclized product **9** is

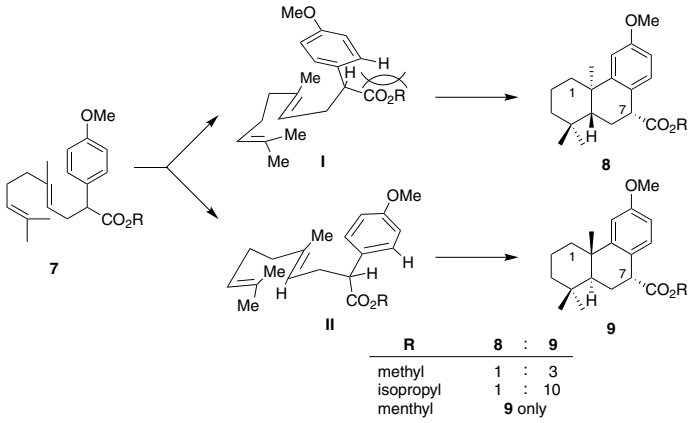

Fig. 3. Stereo-selectivity in the cyclization of modified polyenes.

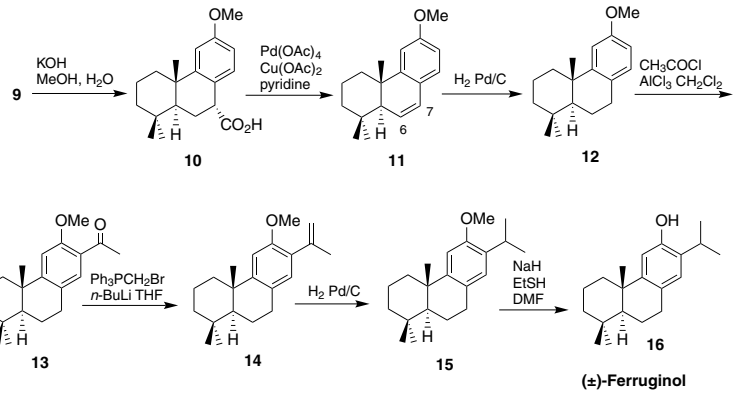

Fig. 4. Synthesis of (±)-ferruginol.

shown in Fig. 4. The ester group of **9** was hydrolyzed to give the carboxylic acid **10**, which was then heated with $Pb(OAc)_4$-$Cu(OAc)_2$ in pyridine to form the 6,7-unsaturated compound **11**. Compound **11** was hydrogenated to give tricyclic methyl ether **12**. The isopropyl group was introduced on the aromatic ring in three steps, Friedel-Crafts acylation, Wittig reaction, and hydrogenation, to give ferruginol methyl ether (**15**). Methyl ether **15** was treated with ethanethiol and sodium hydride in N,N-dimethylformamide (DMF) at 120 °C for 2 days to give (±)-ferruginol (**16**).

(+)-Ferruginol (**22**) was synthesized from the acid **17**, which was treated with (*S*)-BINOL, DCC, and DMAP to give (*S*)-BINOL ester **18**. The chiral ester was converted to **20** using LDA, HMPA, and geranyl chloride (**19**) in 95% de. The (*S*)-BINOL ester **20** was treated with $BF_3{\cdot}OEt_2$ in nitromethane to afford cyclized BINOL ester **21** stereoselectively. The ester **21** was converted to (+)-ferruginol **22** by procedures similar to those for racemic ferruginol (**16**) (Fig. 5). (−)-Ferruginol (**23**) was synthesized using (*R*)-BINOL *via* similar reactions.

4. Synthesis of (±)-totarol

The totarane skeleton has an isopropyl group at C14, whereas abietane has an isopropyl group at C13. A typical totarane compound, totarol[22] (**29**), was synthesized as a racemate from another modified polyene (**25**) by a similar cyclization[23] (Fig. 6). 2-Methoxy-6-methylbenzoic acid (**24**) was treated with LDA and then geranyl chloride to give the acid (**25**), which was methylated with CH_3I and K_2CO_3 in CH_3CN to afford a modified polyene methyl ester **26**. Cyclization of the modified polyene **26** with procedures similar to those for ferruginol ($BF_3{\cdot}OEt_2$-nitromethane) gave a mixture of **27** and **28** in a 1:3 ratio. The methyl ester of **28** was transformed into an isopropenyl group and the methyl ether of the product was treated with BBr_3 and then hydrogenated to give (±)-totarol (Fig. 6).

5. Syntheses of highly oxidized diterpenes with an abietane skeleton and their anti-MRSA and anti-VRE activities

The oxidized abietane diterpenes, abietaquinone methide (**1**), taxodione (**2**), 6,7-dehydroferruginol methyl ether[24] (**11**), ferruginol (**15**), royleanone[13] (**30**), demethylcryptojaponol[25] (**31**), salvinolone[26,27] (**32**), sugiol methyl ether[28,29] (**33**), sugiol[24,30,31] (**34**), 5,6-dehydrosugiol methyl ether (**35**), 5,6-dehydrosugiol (**36**), and 6β-hydroxyferruginol (**37**) were synthesized from dehydroferruginol methyl ether (**11**) as racemates *via* the three routes **A**, **B**, and **C**[32] (Fig. 7).

The three routes can be described briefly as follows. In the route **A**, ferruginol was oxidized at the *ortho*-position of the phenol, and the catechol monoester produced was introduced into abietaquinone methide **1** (*via* reduction and auto-oxidation), royleanone (**30**) (*via para*-oxidation at C14), demethylcryptojaponol (**31**) and salvinolone (**32**) (*via* oxidation at the C7, benzylic position) using protecting groups. In the route **B**, dehydroferruginol methyl ether (**11**) was oxidized by hydroboration and Jones' oxidation to give sugiol methyl ether (**33**), whose methyl ether was deprotected with EtSH and NaH in DMF to afford sugiol (**34**). Introduction of a double bond

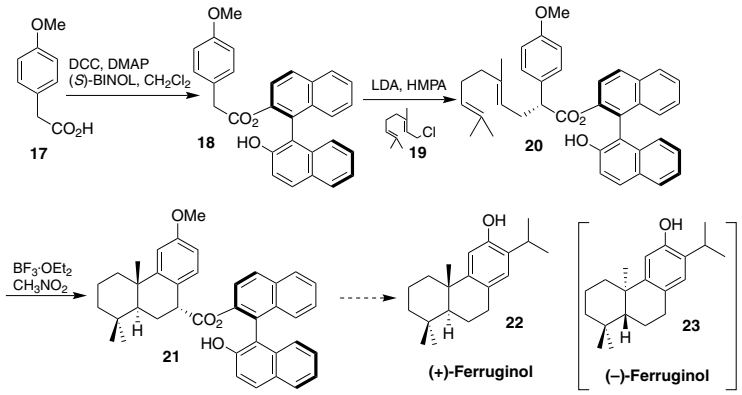

Fig. 5. Synthesis of (+)-ferruginol.

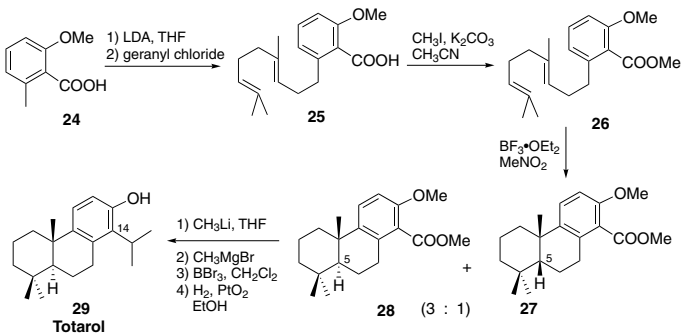

Fig. 6. Synthesis of totarol.

at C5-6 of **11** produced 5,6-dehydrosugiol methyl ether (**35**) and 5,6-dehydrosugiol (**36**). In the route **C**, dehydroferruginol methyl ether (**11**) was oxidized with *m*-chloroperbenzoic acid (*m*CPBA) and then treated with TsOH to give 6-oxoferruginol methyl ether, which was converted into 6β-hydroxyferruginol (**37**) via deprotection of the methyl group dollowed by LAH reduction. 6β-Hydroxyferruginol (**37**) was converted into taxodione (**2**) and 12-hydroxyabieta-8,11,13-trien-6-one (**38**). 12-Hydroxyabieta-8,11,13-trien-6-one (**38**) is not a natural diterpene, but was synthesized for its biological interest.

6. Antibacterial activity of synthesized diterpenes against antibiotic-resistant bacteria

Multiple drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococcus (VRE), cause serious infections in hospitals worldwide. Antimicrobial compounds against antibiotic-resistant bacteria can be found in natural resources. Many natural phenolic diterpenes show antibacterial activity. The anti-MRSA (664, 730, 996) and anti-VRE (VanA, VanB, VanC) activities of the synthesized phenolic diterpenes and related compounds [32] were examined and revealed 6 compounds with relatively potent activity (Table 1). 11-Hydroxy-12-oxo-

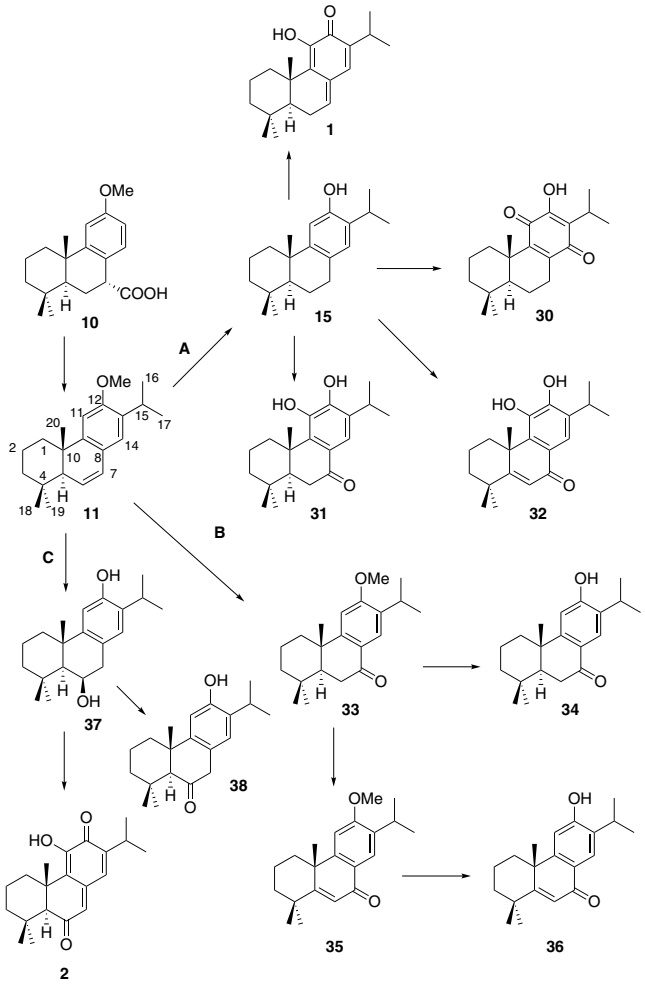

Fig. 7. Syntheses of highly oxidized diterpenes with an abietane skeleton.

Table 1. Minimum inhibitory concentration (MIC, μg/mL) of synthesized phenolic diterpenes against MRSA and VRE

	Strains, MIC (μg/mL)					
compounds	MRSA			VRE		
	MRSA996	MRSA730	MRSA664	VanA	VanB	VanC
1	1	1	0.5	0.5	1	0.5
31	4	4	6	8	8	8
32	6	6	8	16	16	16
38	4	4	4	4	6	6
37	8	8	8	8	16	16
2	10	8	8	4	6	4
Vancomycin	2	2	2	256	128	16
(±)-Ferruginol (**16**)			125			62.5
(+)-Ferruginol (**22**)			> 125			> 125
(–)-Ferruginol (**23**)			62.5			31.3

7,9(11),13-abietatriene (abietaquinone methide, **1**) possessed the most potent activity with a minimum inhibitory concentration (MIC) of 0.5-1.0 μg/mL, which is more potent than the activity of vancomycin (Table 1). Taxodione (**2**, MIC: 4-10 μg/mL) also showed potent activities against both types of bacteria.

The synthetic ferruginol, a well known antibacterial diterpene, racemic (±)-**16**, natural (+)-**22**, and unnatural (−)-ferruginol (**23**), showed weak anti-MRSA and anti-VRE activities.

The results suggest the potential of catechol and quinone methide diterpenes as novel antibacterial compounds. Thus, the facile synthesis of optically active diterpene catechols for further investigation of their various biological activities is planned.

7. *Ortho*-oxidation of phenols

Many natural diterpene phenols have been isolated from popular plants. *ortho*-oxidation of phenols was expected to provide effective synthesis of diterpene catechols from natural diterpene phenols. Benzoyl peroxide (BPO) was used in our first synthesis of abietaquinone methide **1**, by *ortho*-oxidation of (±)-ferruginol (**15**).[32] However, big explosions were reported due to BPO in some industrial plants. We thus planned to use another reagent for the *ortho*-oxidation reaction. Benzeneseleninic anhydride is known an effective reagent for *ortho*-oxidation reaction,[33] but this reagent was not suitable for the synthesis because of toxicity. Therefore, 2-iodoxybenzoic acid (IBX) was examined for the *ortho*-oxidation of ferruginol; however, it produced a complex mixture. Kubota and Takeuchi reported the unexpected formation of *meta*-chlorobenzoyl peroxide (*m*CBPO) (**39**) by heating *m*CPBA in dimethyl formamide (DMF), which was accompanied by an explosion.[34] The formation of *m*CBPO after the explosion indicates that *m*CBPO is stable at high temperatures. In fact, solid *m*CBPO melts at temperatures higher than 110 °C with foaming when heated in a glass tube. Therefore, reaction of phenols with mCBPO was attempted at the reflux temperature of CH_2Cl_2 and $CHCl_3$. Phenols were safely oxidized with *m*CBPO at the reflux temperature in $CHCl_3$.

7-1. *Ortho*-oxidation of phenols using *m*CBPO

Diacylperoxides were synthesized from the corresponding carboxylic acids by treatment with dicyclohexylcarbodiimide (DCC) to form the adduct followed by addition of m-chloroperbenzoic acid (*m*CPBA) to give asymmetric diacylperoxides (Fig. 8). *m*CBPO (**39**) was synthesized from *m*CBA using a similar procedure.[11] The prepared diacylperoxides were examined for suitability for *ortho*-oxidation of phenols without separation. After the reaction, the mixture was reduced with $LiAlH_4$ (LAH) and then acetylated with acetic anhydride and pyridine (Table 2) .[35] The products were separated for identification and determination of yields. The solution of phenol and *m*CBPO in $CHCl_3$ was heated at the reflux temperature for 16 h and then reduced with LAH. The reaction produced catechols from the corresponding phenols with moderate yields. *m*CBPO (**39**) crystallized easily from the reaction mixture and was stable under the reaction conditions for *ortho*-oxidation of phenols to give moderate yields of catechols.

*m*CBPO was synthesized easily from the commercially available peroxide *m*CPBA. Table 3 shows the stabilities of benzoyl peroxide (BPO) and the chlorinated derivatives.[36] The decomposition temperature and ignition temperature of *m*CBPO and *p*CBPO were higher than those of BPO and *o*CBPO, whereas the heat of decomposition of *m*CBPO and *p*CBPO were lower than those of BPO and *o*CBPO.

These data and the availability of *m*CBPO prompted its

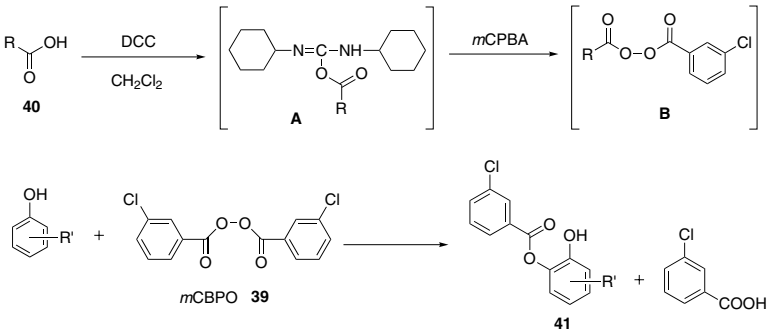

Fig. 8. Oxidation of phenols with *m*CBPO.

Table 2. Oxidation of phenols with *m*CBPO.

entry	Phenol	Yield of catechol[a]
1	*o*-Cresol	n. r.
2	*m*-Cresol	35 (4-Me)
3	*p*-Cresol	75 (4-Me)
4	2,3-Xylenol	11 (3,4-diMe)
5	3,4-Xylenol	39 (3,4-diMe : 4,5-diMe = 1 : 3)
6	3,5-Xylenol	33 (3,5-diMe)
7	2,4-Xylenol	n. r.
8	3,4,5-Trimethylphenol	56 (3,4,5-tri-Me)
9	4-Isopropylphenol	29 (4-*i*-Pr)
10	4-*tert*-Butylphenol	17 (4-*t*-Bu)
11	2,4-Di-*tert*-butylphenol	n. r.

[a] Estimated by ^{1}H-NMR of the diacetate. n.r. : no reactio n

use as the reagent for *ortho*-oxidation of phenols. Kubota determined that the explosion of *m*CBPO in DMF occurred at a temperature higher than 125 °C by differential thermal analysis.[34] Therefore, *m*CBPO was treated in CH_2Cl_2 or $CHCl_3$ at the reflux temperature.

8. Syntheses of diterpene catechols from plants constituents

Various diterpene catechols were efficiently synthesized from natural diterpene phenols using the *ortho*-oxidation reaction described here. Catechol diterpenes can be classified into abietane, totarane, and podocarpane compounds by their carbon skeleton (Fig. 11). The three skeletons have a similar three-ring system, but the abietane skeleton has an isopropyl group at C13, the totarane skeleton has an isopropyl group at C14, and the podocarpane skeleton contains no isopropyl group. Abietaquinone methide **1** was synthesized first from dehydroabietic acid, which is used as an additive of plastics and paper.[11] Ferruginol (**22**) was used as an intermediate in the synthesis of **1**. After the synthesis, it was discovered that **22** could be obtained efficiently from the resin of the bark of *Cryptomeria japonica* (Japanese name: sugi). Totarol (**42**), the starting compound for syntheses of catechols with a totarane skeleton, could be isolated from fresh leaves of *Thujopsis dolabrate* (Japanese name: hiba) in about 0.2% yield. Catechols with the podocarpane skeleton were synthesized from ferruginol, **22**, by removing the isopropyl group. Details of the syntheses of catechols have been reported previously.[37]

8-1. Syntheses of catechols with a totarane skeleton

Natural totarol (**42**) was oxidized with *m*CBPO in CH_2Cl_2 to give an *ortho*-oxidized catechol ester (**43**) and its isomer (**44**) through an ester exchange reaction. The mixture of catechol esters was reduced with LAH to give a catechol (**45**). Catechol **45** was stable in air and was oxidized with Ag_2O to give an *ortho*-quinone (**46**). The ^{1}H-NMR spectrum showed that the content of *ortho*-quinone methide **47** was about 1% and that the tautomeric isomerization of **46** to **47** was very slow. The product ratio of **46** and **47** varied (0:100 ~ 1:7) by reaction time and method of silica gel chromatography. The isolated quinone methide **47** was oxidized on silica gel in air to produce maytenoquinone (**5**).[23,37] Totarane compounds, catechol **45**, quinone **46**, and quinone methide **47** were stable in air, at room temperature during column chromatography, and during identification by NMR in $CDCl_3$. In contrast, the catechol with the abietane skeleton was easily oxidized in air to quinone **46**, which was isomerized to abietaquinone methide (**1**). The difference in carbon skeleton of totarane and abietane is the position of the isopropyl group, but the stabilities of the catechol, quinine, and quinone methide were very different.

8-2. Syntheses of catechols (52, 53, 56) with a podocarpane skeleton

The podocarpane skeleton contains no isopropyl group on the C-ring, in contrast to the abietane and totarane skeletons. The podocarpane skeleton was prepared from an abietane compound by removing the isopropyl group with *ipso*-

Table 3. Stability of benzoyl peroxide (BPO) and chlorinated derivatives.

Peroxide	Decomposition temp. Initial	Peak (°C)	Heat of decomposition (kJ/mol)	Ignition temp. (°C)
BPO	107	108	300	106
*m*CBPO	112	121	245	114
*o*CBPO	89	102	270	87
*p*CBPO	129	135	190	127

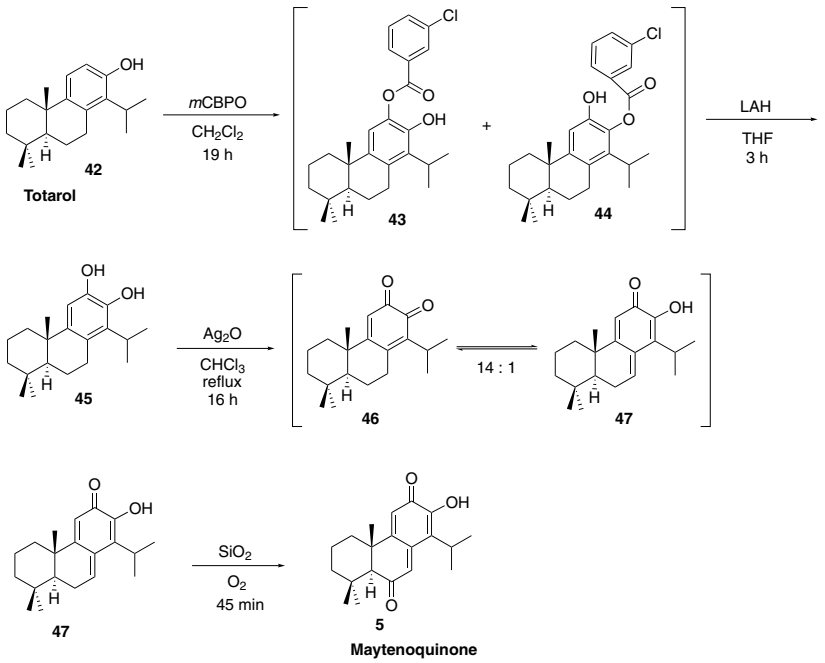

Fig. 9. Synthesis of maytenoquinone.

substitution under Friedel-Crafts acylation. Ferruginol methyl ether was treated with AcCl and $AlCl_3$ to afford nimbosone (**49**) in 73% yield. Nimbosone (**49**) was treated with *m*CPBA and TsOH to give the phenol **51** which was formed by hydrolysis from ester **50**, the product of Baeyer-Villiger reaction of **49**. Deprotection of the methyl ether of **51** gave podocarpa-8,11,13-triene-12,13-diol (**52**) with the isomer (**53**) by a rearrangement. The phenol **51** was converted into dimethyl ether **54**, which was oxidized at C-7 (benzylic position) with CrO_3 to give dimethyl ether **55**. The dimethyl ether of **55** was treated with BBr_3 to give nimbidiol (**56**).[38]

9. Antimicrobial activity of synthesized diterpene catechols and quinone methides

The antibacterial activities of the diterpene catechols synthesized were examined.[37]

Skin diseases such as acne vulgaris are caused by the proliferation of various bacteria, especially *Propionibacterium acnes* and *S. aureus*, on the skin. The lack of effective treatments for skin diseases such as acne vulgaris necessitates the development of new types of antibacterial agents.

Minimum inhibitory concentration (MIC) of the

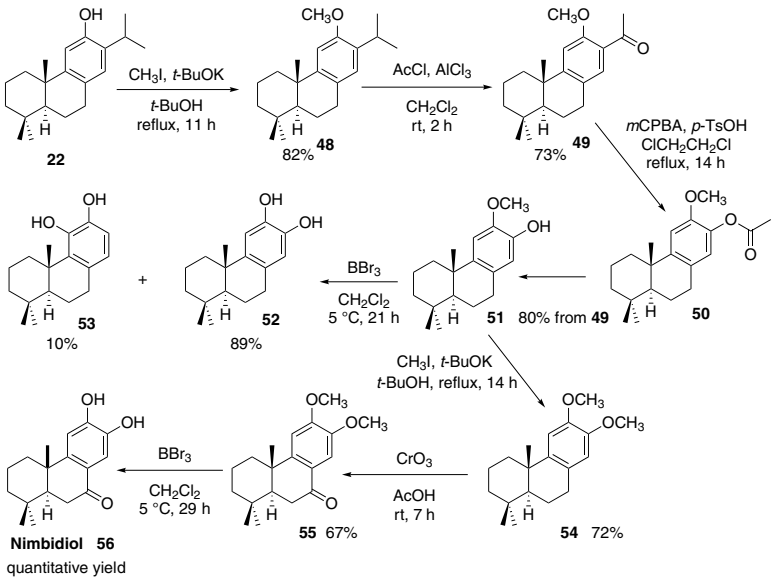

Fig. 10. Synthesis of nimbidiol.

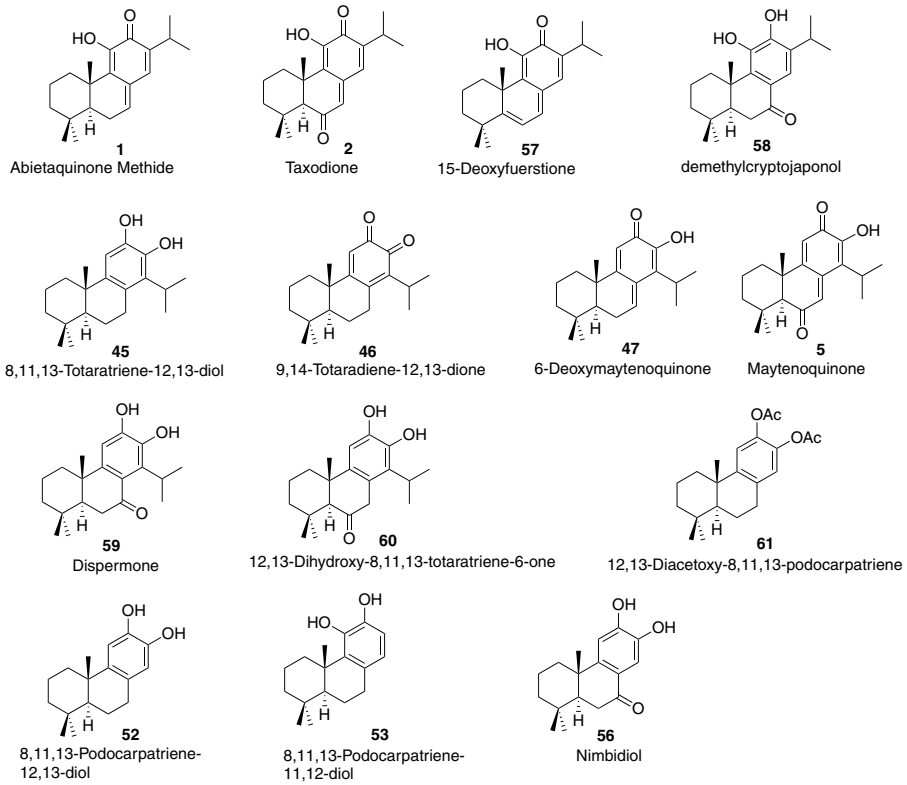

Fig. 11. Synthesized phenolic diterpenes with an abietane, totarane, or podocarpane skeleton, and related compounds.

synthesized diterpenes, abietane, totarane, and podocarpane compounds were evaluated against *P. acnes* (ATCC 6919) and *S. aureus* ME/GM/TC resistant (ATCC33592) (MRSA). The MICs of ampicillin and vancomycin also were measured as reference compounds against *P. acnes* (ATCC 6919) and MRSA, respectively. Four abietane derivatives (**1**, **2**, **57**, **58**) and four totarane derivatives (**45**, **46**, **47**, **5**) showed potent or moderate antibacterial activity against both *P. acnes* and MRSA, whereas podocarpane catechols (**61**, **52**, **53**, **56**) showed moderate activity (Table 4 and Fig. 11). Diacetates of diterpene catechols possessed less potent antibacterial activity compared to the other catechol derivatives. The MIC (1 μg/mL) of abietaquinone methide (**1**) and 8,11,13-totaratriene-12,13-diol (**45**) was comparable to vancomycin toward *S. aureus* (MRSA). Toxicity of **1** was evaluated by oral dose in mice. No serious change in body weight and behavior of the mice was observed for 7 days after the oral dose of 1000-2000 mg/kg of **1**.

In this research, ferruginol (**22**), the major constituent of the resin of *C. japonica* bark and totarol (**42**), the major constituent of *T. dolabrata* leaves, readily available starting materials were used for the syntheses. The effective use of these plant resources may help contribute to the preservation of Japanese forests.

10. Syntheses of carnosic acid and carnosol, anti-oxidants in rosemary from pisiferic acid

Rosemary is a herb in the *Salvia* family used in cooking and for folk medicines; it contains many anti-oxidant diterpenes.[39,40] The German Commission E Monographs consider rosemary extracts effective for treating indigestion and increasing blood circulation. Recently, major antioxidants in rosemary, carnosic acid (**64**) and carnosol (**65**), have attracted attention for their neuron-protective effects.[41] Many studies have reported biological activities of **64** and **65**, such as neuron-protective activity, nerve growth factor forming promoters, and therapeutic agents for amnesia, dementia, Alzheimer's disease, and lipid absorption inhibition.[42-44] Carnosic acid is available commercially, but is very expensive (>$630/500 mg) and is isolated by extraction from natural rosemary. Despite the difficulty of isolating carnosic acid (**64**) and carnosol (**65**), no efficient method for synthesizing carnosic acid is available.

Chamaecyparis pisifera (Japanese name: sawara) is a tree in Japan with leaves that contain the phenolic diterpene, pisiferic acid (**62**), as a major constituent.[45] Pisiferic acid (**62**) has an abietane carbon skeleton, and can be described as carnosic acid (**64**) with a carboxyl group at C-10 and a phenolic hydroxyl group at C-12. Carnosic acid (**64**) has an additional phenolic hydroxyl group at C11. Therefore, carnosic acid was synthesized from pisiferic acid by *ortho*-oxidation of the 12-hydroxyl group.

Leaves of *C. pisifera* were collected on the Fuchu campus of Tokyo University of Agriculture and Technology. Fresh leaves were extracted with methanol under reflux for 24 h. The extract was evaporated and the residue was extracted with ethyl acetate and water. The organic layer was evaporated and the residue was chromatographed on a short column of silica gel using hexane-ethyl acetate (3:1). Crystallization from ethyl acetate-hexane gave white crystals of pisiferic acid (**62**). The procedure for the separation of **62** was simple and yielded **62** in about 0.6% based on fresh leaves.

As described above, diterpene catechols were synthesized using *ortho*-oxidation of phenols with *m*CBPO. Synthesis of carnosic acid (**64**) from pisiferic acid (**62**) was examined first using *ortho*-oxidation by *m*CBPO. Pisiferic acid (**62**) was oxidized with 3 molar equivalents of *m*CBPO in CH_2Cl_2 for 16 h at ambient temperature. The crude product **63** was hydrolyzed with sodium hydroxide and sodium borohydride ($NaBH_4$) in methanol. The products were separated by chromatography to give **64** in 11% yield from **62**. The spectral properties (^{1}H- and ^{13}C-NMR) of synthetic carnosic acid (**64**) were identical to those of the natural carnosic acid.[39,40] The yield of carnosic acid after oxidation with *m*CBPO was greater than 50% by ^{1}H-NMR spectroscopy; however, the isolated yield was very low (11%) due to the separation difficulty of **64** from *m*CBA (Fig. 12).

Pisiferic acid (**62**) was then oxidized with 2-iodoxybenzoic acid (IBX) in $CHCl_3$-CH_3OH at ambient temperature for 1 h under argon. The product was assumed to be the unstable *ortho*-quinone, therefore it was reduced by $NaBH_4$ under argon for 4 h without isolation. The 2-iodobenzoic acid was easily crystallized in hexane, the supernatant was evaporated, and the residue was chromatographed to afford **64** in 72% yield from **62** with sequential two step reactions in one pot.

Carnosic acid (**64**) was then converted to carnosol (**65**) in 63% yield by oxidation with Ag_2O.[46,47]

This synthesis of carnosic acid (**64**) has been conducted successfully on a large scale and is now available commercially as a reagent from a Japanese company.

Minimum inhibitory concentrations (MIC) of the synthetic carnosic acid (**64**) and carnosol (**65**) were measured against *P. acnes* (ATCC 6919)[48] and *S. aureus* ME/GM/TC resistant (ATCC 33592)[49] to show the potential of these compounds as

Table 4. Minimum inhibitory concentration (MIC, μg/mL) of synthesized abietane, totarane, and podocarpane diterpenes against *P. acnes*.

Compound	MRSA	*P. acnes*
1	1	1
2	10	10
57	10	3
58	10	X
45	1	1
46	3	1
47	3	X
5	1	3
59	10	X
60	3	30
61	>100	>100
52	3	10
53	10	10
56	30	X
Vancomycin	1	X
Ampicillin	X	0.1

X : not measured

antibacterial drugs. Both synthetic compounds possessed potent antibacterial activity against *P. acnes* and *S. aureus* (Table 5). The anti-*P. acnes* activity of synthetic carnosic acid (**64**) (1 μg/mL) was more potent than that of natural carnosic acid (16 μg/mL) as reported by Weckesser.[50] Because different species of *P. acnes* might have been used in the two studies, comparison of the antibacterial potency of the two carnosic acids is difficult. The isolation and purification of carnosic acid (**64**) and carnosol (**65**) from plants are very difficult in general.[3] Our synthesis affords pure carnosic acid in reasonable yield. The MICs of the antibiotics ampicillin and vancomycin were measured against *P. acnes* (ATCC 6919) and *S. aureus*, respectively, for reference.

The toxicity of pisiferic acid (**62**) and synthesized carnosic acid (**64**) was evaluated by providing an oral dose to mice.[51] No significant change in body weight or behavior of the mice was observed for 7 days after the oral dose of 1000–2000 mg/kg of **64**. Pisiferic acid (**62**) produced slight toxicity after an oral dose of 2000 mg/kg. Because carnosic acid is a constituent of some food items, no serious toxicity was expected.

The bioactivities of synthetic carnosic acid (**64**) are not fully understood and further research needs to be done. Because humans have historically utilized rosemary in various ways, it should have potential as various utilities, *e.g.* an external treatment for acne, cosmetics and nutritional therapies, as well as anti-inflammatory agents, antioxidants, and food additives. Since it is a synthesized natural product, governmental approvals are needed for these applications. The raw material used for production of carnosic acid—the plant *C. pisifera*—is widely distributed in Japan and is easily cultured. Application of these study results may contribute to renewed interest in the forest industry.

11. Conclusions

We succeeded efficient syntheses of various diterpenes of abietane, totarane and podocarpane skeletons with catechol, *ortho*-quinone methide or *ortho*-quinone group from easily available plants constituents. Studies of the biological activities of the synthesized catechols, *ortho*-quinone methides and an *ortho*-quinone showed the potential for various utilities of theses diterpenes. Since diterpenes are relatively common compounds throughout the plant kingdom, their structures are not novel and cutting-edge reactions for their syntheses are not necessary. However, efficient utilization of plants in the chemical industry is important for realizing a sustainable society, and reducing the dependence of society on petroleum as a raw material.

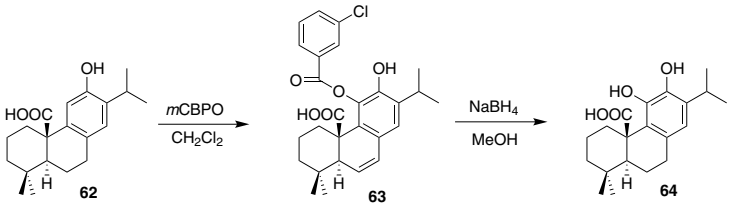

Fig. 12. Synthesis of carnosic acid (**64**) *via* oxidation with *m*CBPO.

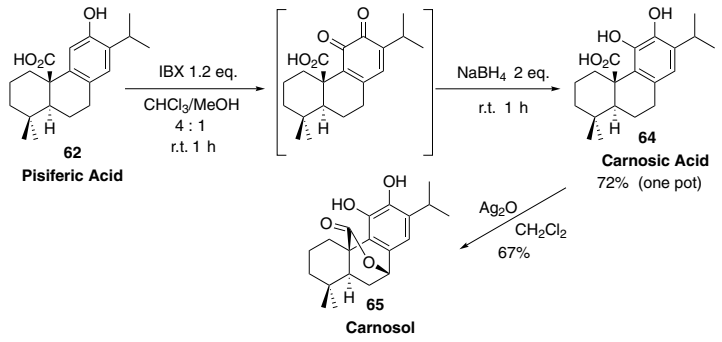

Fig. 13. Syntheses of carnosic acid (**64**) and carnosol (**65**) from pisiferic acid (**62**).

Table 5. Antibacterial activity of synthesized anti-oxidants in rosemary (MIC, μg/mL)

Compound	MRSA	*P. acnes*
Carnosic acid (**64**)	10	1
Carnosol (**65**)	30	10

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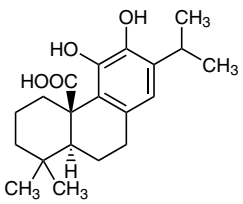
Introduction of the authors :

Masahiro Tada
Professor Emeritus, Tokyo University of Agriculture and Technology

Masahiro Tada received his Ph.D. from University of Tokyo in March 1974 under the supervision of Professor Takeyoshi Takahashi. From April 1974 to September 1975, he worked as a postdoctoral fellow at University of California, Berkley with Professor William G. Dauben. Later, he worked as a research fellow of the Japan Society for the Promotion of Science in the Department of Chemistry, University of Tokyo from April 1976 to March 1977. He was promoted to an assistant professor in the Department of Chemistry in April 1977. He then became a lecturer at Tokyo University of Agriculture and Technology in October 1977 and an associate professor in July 1983. In April 1988, he became a professor. He was appointed as a professor at the Institute of Agriculture and Institute of Symbiotic Technology and Science, Tokyo University of Agriculture and Technology in April 2004. He concurrently served as the director of the University Library from April 2001 to March 2003. He retired from the university in March 2009 and was appointed as Professor Emeritus. He continued his research as a professor at Tokyo University of Agriculture and Technology until November 2009.
His research fields are organic chemistry and natural product sciences. His research interest includes efficient synthesis of bioactive natural products and creation of novel bioactive substances.

TCI Related Compounds



Carnosic Acid
20mg, 100mg [C2488]