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Biological Activities of Phloroglucinol Derivatives from *Eucalyptus* Spp.

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Abstract – *Eucalyptus* is a rich source of biologically active compounds. Among these, phloroglucinol compounds such as sideroxylonals, macrocarpals, euglobals, and robustadials are unique to *Eucalyptus* species. Sideroxylonal A is a very potent attachment-inhibitor. Macrocarpals show very strong antibacterial activity against gram positive bacteria. Macrocarpals also show HIV-RTase inhibitory activity. Euglobals are potent inhibitors of Epstein-Barr virus activation and are developed as skin and antitumor agents. They also show granulation inhibitory activity. In this review we aim to remove the existing confusion in literature on macrocarpals and discuss the biological activities and structure-activity relationships of phloroglucinol compounds.

Keywords – Phloroglucinol, *Eucalyptus* spp., attachment-inhibiting activity, antibacterial activity, HIV-RTase inhibiting activity.

Introduction

Eucalyptus (family-Myrtaceae), native to Australia and well known for its rapid growth, finds extensive use in timber, fuel, and paper pulp industry. Besides, it is a mean to control erosion of soil caused by wind or water and is a source of essential oil whose terpenic components make it useful in medicine and perfumery. The bark and leaves of various species were used to treat colds, influenza, toothaches, snakebites, fever, and diarrhea *etc* (Ghisalberti, 1996). *Eucalyptus* shows unique physiological features to survive in dry land. Most species show distinctive change in features between juvenile and adult stages, which is thought to be controlled by endogenous regulators. In view of rapid growth, *Eucalyptus* plantation has been introduced in many countries and is considered as a potent biomass. Besides the paper and pulp industry, many secondary industries can be developed to recover potentially useful compounds. In recent years, *Eucalyptus* is a subject of active research due to its unique secondary metabolites which show a wide variety of biological activities such as anti-malarial, growth regulatory, antibacterial, HIV RTase inhibitory, antiviral and attachment inhibitory activity against marine sessile organisms responsible for marine fouling (Ghisalberti, 1996 and Singh *et al.*, 1996). Most of these compounds include phloroglucinol derivatives such as robustadials, euglobals, macrocarpals and sideroxylonals. Euglobals and robustadials are cycloadducts of diformyl phloroglucinol and terpenes suggesting the biogenetic cycloaddition of corresponding terpenes and unstable *O*-quinonemethides to generate the chroman

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or spirochroman skeleton of these compounds. Macrocarpals have been suggested to be formed through the cyclization of sesquiterpene precursor initiated by the carbocationic species generated from diformyl phloroglucinol moiety. Sideroxylonals are dimers of diformyl phloroglucinol moiety which can also arise in the same way as euglobals. At the turn of the century, when we are faced with a shortage of natural resources for survey of biologically active compounds, *Eucalyptus* species still offers much promise in this area. The aim of the present review is to lay emphasis on biological activities of these secondary metabolites.

Attachment-inhibitors from *Eucalyptus* spp. – Sideroxylonal A (**1**), which we recently isolated from *E. grandis* was found to be the most potent attachment inhibitor against the blue mussel, *Mytilus edulis galloprovincialis* reported so far (Singh *et al.*, 1996). It showed inhibitory activity at unit 1560 which was slightly higher than 2,5,6-trimethyl gramine (**2**, unit 1360) isolated from marine bryozoan *Zoobotryon pellucidum* (Konya *et al.*, 1994). The activity of sideroxylonal A is almost fifteen times more than that of $CuSO_4$, a standard antifoulant. Grandinal (**3**), a new phloroglucinol dimer showed attachment-inhibiting activity at unit 100 (Singh *et al.*, 1997). The drastic decrease in activity of **3** compared with **1** was attributed to the presence of tautomeric quinoid forms. We also tested macrocarpals isolated (Singh and Etoh, 1995) from *E. amplifolia* and *E. globulus* for attachment-inhibiting activity. Macrocarpals A (**4**), B (**5**), E (**6**), am-1 (**7**), and H (**8**) showed 1-3 times higher activity than $CuSO_4$. The similarity in activity of macrocarpals, which differed from each other in terpene sub-structure, indicated that the diformyl phloroglucinol moiety was responsible for the activity. Grandinol (**9**), a growth-inhibitor having monomeric formyl phloroglucinol structure was far less active than sideroxylonal A, a dimeric diformyl phloroglucinol compound. Grandinol showed activity at unit 8 (Singh *et al.*, 1996). It can be concluded that the presence of a diformyl phloroglucinol moiety may be an essential fea-

Table 1. Attachment-inhibiting (AI) and Antibacterial (AB) Activity of Phloroglucinol Derivatives

Compound	AI (Unit)	AB (MIC, μg/ml)					
		A[a]	B	C	D	E	F
1	1560	3.9[b]	7.8[b]	-[c]	-	-	-
2	1360	-	-	-	-	-	-
3	100	100	50	-	-	-	-
4	300	1.56	1.56	>100	>100	0.78	0.39
5	300	3.13	1.56	>100	>100	3.13	0.78
6	300	0.39	0.78	>100	>100	-	-
7	150	12.8	12.8	-	-	-	-
8	300	-	-	-	-	3.13	0.78
9	8	5	25	-	-	-	-
10	-	0.78	1.56	>100	>100	-	-
11	-	0.78	3.13	>100	>100	-	-
12	-	-	-	-	-	0.39	0.20
13	-	-	-	-	-	1.56	0.39
14	-	-	-	-	-	12.5	1.56
15	-	-	-	-	-	50	6.25
16	-	-	-	-	-	50	6.25
17	-	1.6[b]	-	-	-	-	-

[a]A-*Bacillus subtilis*; B-*Staphylococcus aureus*; C-*Escherichia coli*; D-*Candida albicans*; E-*Streptococcus mutans*; F-*Porphyromonas gingivalis*. [b]Tested by paper disc method (μg/disc). [c]Data not available.

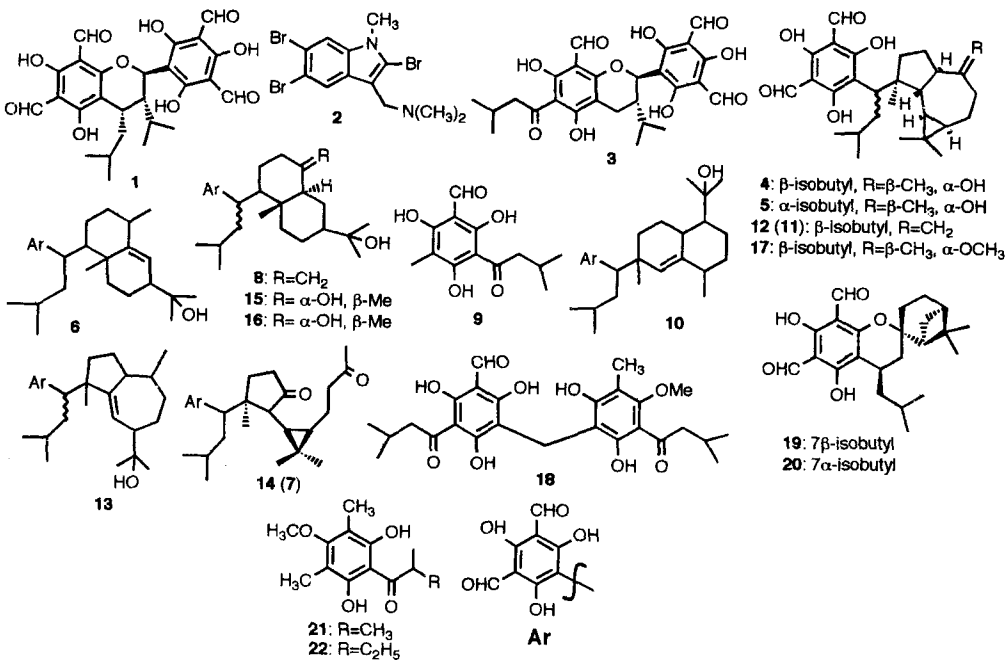

Fig. 1.

ture for attachment-inhibiting activity of these compounds.

Anti-bacterial compounds from *Eucalyptus* spp. – A large number of phloroglucinol compounds isolated from *Eucalyptus* spp. show very strong antibacterial activity. Macrocarpals constitute one such group which show very high activity against gram positive bacteria but are inactive against gram negative bacteria, yeast or fungi. There is a confusion in literature on macrocarpals. Macrocarpals A-G were isolated from *E. macrocarpa* (Yamakoshi *et al.*, 1992). Planar structures were assigned to macrocarpals B (**5**), D (**10**), and G (**11**). The structures to others were not assigned. At the same time, five related compounds M-A (**4**), M-B (**5**), M-C (**12**), M-D (**13**), and M-E (**6**) were isolated from *E. globulus* (Nishizawa *et al.*, 1992). Macrocarpal G (**11**) was assigned same planar structure as M-C (**12**). Diastereomeric relationship between the two compounds is possible. Two structurally different compounds were given similar name macrocarpal D (**10**) and M-D (**13**) by two groups. To remove this ambiguity, we name one of these, M-D (**13**) as macrocarpal X while retaining the original name for the other, macrocarpal D (**10**). We isolated M-E (**6**) from *E. amplifolia* and found that it was identical to an unidentified compound named macrocarpal E (Yamakoshi *et al.*, 1992). The MICs of macrocarpals were in range of 0.78 to 3.13 μg/ml for gram positive bacteria, but were >100 μg/ml against gram negative bacteria, fungi or yeast. These macrocarpals have diformyl phloroglucinol coupled with a sesquiterpene skeleton such as aromadendrane [M-A (**4**), M-B (**5**), M-C (**12**), and eucalyptone (**14**)], eudesmane [M-E (**6**), M-H (**8**), M-I (**15**), M-J (**16**), and M-D (**10**)], and guaiane [M-X (**13**)]. The activity is not altered significantly with changes in sesquiterpene skeleton. This indicates that it may be the formyl phloroglucinol part which is responsible for antibacterial activity. This is substantiated by high activity of grandinol (**9**), which lacks any terpene moiety, against *Bacillus subtilis*. The MIC of grandinol against *B. subtilis* is 5 μg/ml; but against *Streptococcus aureus* it is 25 μg/ml

(Nakayama *et al.*, 1990). The macrocarpals were also tested for anti-bacterial activity against cariogenic bacteria (gram-positive) and periodontopathic bacteria (gram-negative). Macrocarpals A (**4**), B (**5**), C (**12**), D (**10**), and H (**8**) showed strong antibacterial activity at concentrations of 0.20-3.12 μg/ml. Macrocarpals I (**15**), J (**16**), and eucalyptone (**14**) were less active. (Osawa *et al.*, 1996) On these basis, the authors suggested that the antibacterial activity might be regulated by the sesquiter- pene structure and the low activity of M-I (**15**) and M-J (**16**) may be due to presence of two hydroxyl groups. We also isolated a new macrocarpal from *E. amplifolia* which was named macrocarpal-am-1 (**7**). The compound has same planar structure as eucalyptone (**14**) (Osawa *et al.*, 1995). The NMR data of macrocarpal-am-1 in pyridine-d_5 was completely identical to that reported for eucalyptone which suggested that the two compounds may have same stereostructure, as well. We also tested macrocarpal-am-1 against *S. aureus* and *B. subtilis* by paper disc method. The minimum effective dose of macrocarpal-am-1 against these two bacteria was 12.8 μg . We also isolated another compound whose structure was established as 10-methoxy macrocarpal A (**17**) from spectral data. Although 10-methoxy macrocarpal A may be an artifact, but its activity was same as that of macrocarpal A from which it may have been derived. Both inhibited the growth of *S. aureus* and *B. subtilis* at a concentration of 1.6 μg per disc (unpublished results). Dimeric phloroglucinol compounds, sideroxylonals A and B also showed strong antibacterial activity against *S. aureus* and *B. subtilis* (3.9 and 7.8 μg/ml). They also inhibit the growth of Hela cells (Satoh *et al.*, 1992). These compounds also lack terpene moiety which suggests that diformyl phloroglucinol moiety may be responsible for the antibacterial activity.

Macrocarpals also inhibit aldose reductase AR (EC1.1.1.2, alditol: $NADP^+$ oxidoreductase). AR is a target enzyme for the control of diabetic complications such as cataract, retinopathy, neuropathy, and nepharopathy as it catalyses conversion of aldoses to sugars, thereby increasing the concentration of sorbitol and fructose in the tissues. The increased concentration of these sugars leads to diabetic complications. Macrocarpals A (**4**), B (**5**), D (**10**), and G (**11**) inhibited AR in a concentration range of 2.0 to 2.8 μm (I_{50}). Several synthetic phloroglucinol derivatives were also tested for inhibitory activity against AR. The I_{50} values were around 10 μm for diacyl phloroglucinol derivatives whereas monoacyl phloroglucinol derivatives were ineffective. These results suggest that diformyl phloroglucinol moiety was essential for enzyme inhibition. The activity was not affected by chain length of acyl groups as was observed in case of germination inhibitory tests (Murata *et al.*, 1992).

The leaves of *E. robusta* have long been used in Chinese herbal medicine for treatment of dysentery, malaria, and other bacterial diseases. Robustaol A (**18**) and robustadials A (**19**), and B (**20**) were isolated as anti malarial compounds from *E. robusta* (Cheng and Snyder, 1988). The activity of **19** and **20** was higher than **18**.

Recently two new acyl phloroglucinol compounds **21** and **22** were isolated from leaves of *E. robusta* (Cheng and Snyder, 1991). These compounds have structures closely related to grandinol and homograndinol. Both these compounds showed phosphodiesterase inhibitory activity at concentration of 100 and 125 μg/ml (ED_{50}).

Antiviral compounds from *Eucalyptus spp.* – AIDS is the most serious disease today and it is significant that the macrocarpals are also effective inhibitors of HIV-RTase. Among these, M-B (**5**) was most effective RTase inhibitor (IC_{50} 5.3 μm). The IC_{50} for M-A (**4**), M-C (**12**), M-X (**13**) and M-E (**6**) was 10.6, 8.4, 12.0 and 8.1 μm, respectively (Nishizawa *et al.*, 1992).

Table 2. Relative ratio[a,b] of EBV-EA Activation with respect to Positive Control (100%) in presence of Euglobals and Related Compounds

Compound	Concentration[c]							
	1000		500		100		10	
23	18.2	(70)	27.3	(>80)	51.5	(>80)	85.1	(>80)
24	13.6	(60)	43.7	(>80)	78.5	(>80)	100.0	(>80)
25	7.8	(>80)	62.2	(>80)	89.5	(>80)	100.0	(>80)
26	-[d]	(0)	0.0	(20)	31.5	(>80)	100.0	(>80)
27	-[d]	(0)	0.0	(20)	38.9	(>80)	89.5	(>80)
28	0.0	(20)	0.0	(40)	73.6	(70)	100.0	(>80)
29	0.0	(50)	15.6	(>80)	70.3	(>80)	100.0	(>80)
30	0.0	(60)	25.4	(>80)	73.3	(>80)	100.0	(>80)
31	0.0	(70)	26.2	(>80)	68.4	(>80)	100.0	(>80)
32	0.0	(70)	0.0	(>80)	55.7	(>80)	93.8	(>80)
33	0.0	(70)	15.3	(>80)	45.2	(>80)	79.6	(>80)
34	0.0	(60)	28.9	(>80)	80.5	(>80)	100.0	(>80)
35	0.0	(20)	0.0	(30)	59.1	(>80)	100.0	(>80)
36	0.0	(10)	0.0	(20)	68.7	(>80)	91.3	(>80)
37	0.0	(20)	11.4	(30)	70.2	(>80)	100.0	(>80)
38	0.0	(20)	-[e]		0.0	(70)	86.0	(70)
39	0.0	(0)	-[e]		0.0	(60)	78.5	(>80)
40	0.0	(40)	-[e]		60.2	(60)	100.0	(>80)
41	36.8	(50)	-[e]		100.0	(>80)	100.0	(>80)
42	10.5	(40)	-[e]		71.5	(60)	100.0	(>80)
43	70.0	(60)	-[e]		88.9	(>80)	100.0	(>80)
44	0.0	(40)	-[e]		5.3	(70)	81.2	(>80)

a) Values represent relative percentages to the positive control value (100%). b) Values in parentheses are viability percentages of Raji cells. c) Mol ratio/TPA (20ng≈32pmol/ml). d) Not detected. e) Not tested.

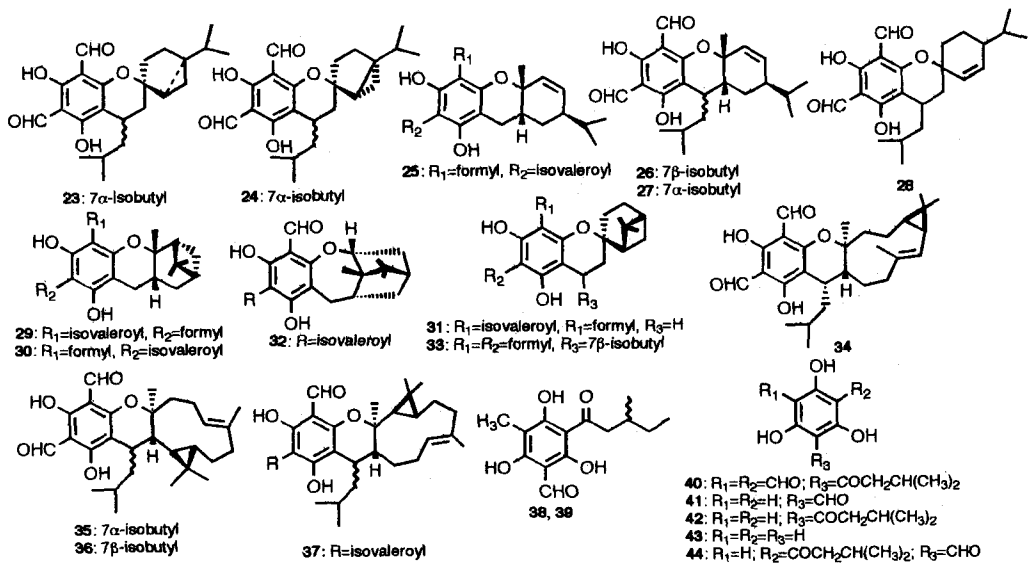

Fig. 2.

Euglobals constitute the most important and the largest group of phloroglucinol compounds with antiviral activity. To date, a large number of euglobals, compounds with

phloroglucinol-monoterpene (sesquiterpene) structures have been isolated from *E. globulus, E. tereticornis, E. incrasata, E. grandis* and *E. amplifolia*. Some of the euglobals show strong inhibition of Epstein-Barr virus (EBV) activation induced by tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (Takasaki *et al.*, 1990 and 1995). Among euglobals with monoterpene structure, euglobals Ib (**23**) and IIa (**24**) showed strong inhibition at 1000 mol/TPA ratio. These compounds also showed moderate activity at 500 molar ratio to TPA. Euglobal IIc (**25**) also showed considerable activity at 1000 and 500 mol/TPA ratio. Euglobals $1a_1$ (**26**) and $1a_2$ (**27**) showed strong activity at 100 molar ratio per TPA but were also highly toxic to Raji cells at 1000 and 500 molar ratio per TPA. Euglobal IIb (**28**) was also highly toxic to Raji cells. Euglobals G-1 (**29**), G-2 (**30**), G-4 (**31**), G-5 (**32**), and Am-2 (**33**) exhibited 100% inhibition at 1000 mol ratio/TPA, and more than 70% inhibition at 500 molar ratio. They also showed slight inhibition even at 100 molar ratio/TPA. Furthermore these compounds also preserved high viability of Raji cells even at high concentration. Among euglobals with sesquiterpene structure, euglobal III (**34**) showed strong inhibitory activity at 100 mol/TPA ratio. Euglobals IVa (**35**), IVb (**36**), and VII (**37**) showed strong cytotoxicity on Raji cells (< 30% viability at 500 mol/TPA). Euglobals **29-34** were more active than Glycyrrhetic acid, a known strong anti-tumor-promotor. A few terpenoids and synthetic acyl-phloroglucinol compounds were also tested for inhibition on EBV-EA activation. The monoterpenoids or the sesquiterpenoids lacked any inhibitory activity which indicated that presence of acyl phloroglucinol is essential for the activity. Out of a large number of acyl phloroglucinol compounds tested, those with moieties closely resembling euglobals, such as Grandinol (**9**), (*S*)-homograndinol (**38**), and (±)-homograndinol (**39**) showed strong inhibition of EBV-EA activation at 100 mol/TPA. But these compounds showed very high toxicity at 1000 mol/TPA. Compound **40** which has an isovaleroyl group on aromatic ring showed similar activity to euglobal IIc (**25**) and euglobal VII (**37**). Compound **41** which lacked isovaleroyl group showed lesser activity. It was also observed that both formyl and carbonyl group are necessary for activity as removal of any one group (**41** and **42**) or both the groups (**43**) results in drastic loss of activity. Aromatic methyl group of grandinol was not necessary as its removal (**44**) did not affect activity. It was concluded that:

1. Euglobals with monoterpene structure are more active
2. Acyl phloroglucinol moiety is essential
3. Two acyl groups are required
4. Chain lengths on acyl groups affect activity where as chain length of aromatic alkyl group is insignificant

Conclusion – A variety of formyl and acyl phloroglucinol compounds have been isolated from various species of *Eucalyptus*. These compounds exhibit interesting biological activities. Sideroxylonal A and macrocarpals show strong inhibitory activity against the blue mussel, *Mytilus edulis galloprovincialis*. In fact sideroxylonal A, which is a dimer of diformyl phloroglucinol, is the most potent inhibitor isolated so far. Macrocarpals having one diformyl phloroglucinol moiety are less active than sideroxylonal A. Lesser activity of grandinal, also a dimeric diformyl phloroglucinol, was attributed to presence of quinoid forms. Macrocarpals show very strong activity against gram positive bacteria and also slight activity to inhibit HIV-RTase. Euglobals inhibit activation of EBV-EA induced by TPA. It has been shown that acyl phloroglucinol moiety is indispensable for these compounds to show this activity. It can be concluded that *Eucalyptus* is a source of biologically active compounds and deserves further attention. Furthermore, the biological activities of these compounds arouse

special interest to attempt their synthesis, and in fact some reports on the synthesis of robustadials and euglobals have appeared (Chiba *et al.*, 1995 and 1996, Saloman *et al.*, 1988, Koser and Hoffman, 1993, Majewski *et al.*, 1994, Aukurst and Skattebφl, 1996).

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