132. Colchicine Models: Synthesis and Binding to Tubulin of Tetramethoxybiphenyls

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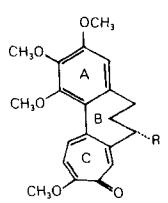
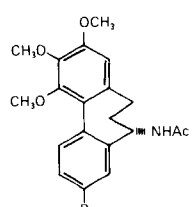
Synthesis of tetramethoxybiphenyl **21** was accomplished from 4-phenylcyclohexane-1,3-dione **13** by aromatization to biphenyl **19** and reductive removal of the phenolic OH group as phenyltetrazolyl ether. Tetramethoxybiphenyls **34** and **40** were obtained from 4-phenylcyclohexenone **26** *via* ester **27**. The tetramethoxybiphenyls **21**, **34**, and **40**, and analogs **28**, **29**, and **31** were evaluated for antitubulin activity and as antimitotic agents with *L1210* murine leukemia cells. Compounds **31** and **34** had significant effects on the *in-vitro* polymerization of tubulin. Compound **31** was the most cytotoxic of the six new biphenyls studied (IC_{50} for cell growth, 0.6M) and caused the accumulation of cells in metaphase arrest.

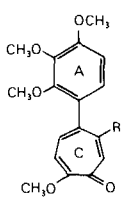
Introduction. – The antimitotic effect of colchicine and its inhibition of migration and phagocytosis of polymorphonuclear leukocytes are attributed to its binding to tubulin, which disorients the structural organization of microtubules [1–4]. Data collected from studies of many colchicinoids (compounds chemically related to colchicine) in assays, measuring binding to tubulin *in vitro* an antitumor activity *in vivo* [4] [5], and inhibition of



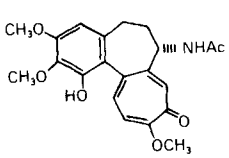
1 R = NHAc
2 R = H



3 R = $COOCH_3$
4 R = OCH_3



5 R = H
6 R = Cl



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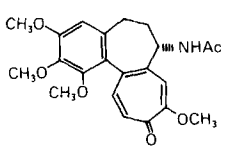

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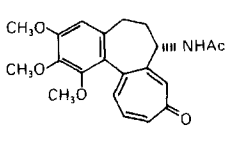

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[1]) On sabbatical leave from the *Fujisawa Pharmaceutical Co. Ltd.*, Tsukuba City, Japan.

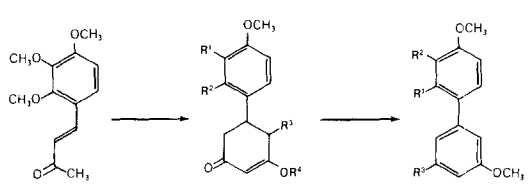
amyloid synthesis [6], showed that all the compounds active in bioassays did bind well to tubulin. High affinity for the protein is shown by the compounds including colchicine (**1**), deacetamidocolchicine (**2**), allocolchicine (**3**), *N*-acetylcolchinol *O*-methyl ether (**4**), and synthetic phenyltropolones **5** and **6** [4] [7] [8]. Colchicinoids of the natural series which do not bind well to tubulin are 1-demethylcolchicine (**7**) [5], isocolchicine (**8**) [9], and colchicide (**9**) [10].

It has been recently shown that optical isomers of colchicinoids [11] have the (a*R*,7*R*)-configuration which is not accepted by the protein [12]. Comparison of active with inactive colchicinoids derived from colchicine, furthermore, reveals that inactive compounds either lack one of the four MeO groups (**7**, **9**) or have it displaced as in **8**. To test the hypothesis that the presence of four strategically located MeO groups is important for binding to tubulin of colchicinoids prompted us to prepare tetramethoxybiphenyls **21**, **34**, and **40** with three MeO groups similarly positioned in one of the two aromatic rings as in colchicine, and a fourth MeO group attached to the second Ph ring in the 2′-, 3′-, or 4′-position. Also prepared were analogs **28**, **29**, and **31**, having an additional substituent at C(2′), expected to hinder free rotation of these tetramethoxybiphenyl molecules.

We thought that contraction of the 7-membered tropolone ring in **5** and **6** to a 6-membered ring in **3** and **4** was a justifiable molecular simplification, and that a comparison of tetramethoxybiphenyls **21**, **34**, and **40** in the tubulin binding assay would permit assessment of the validity of this hypothesis. Our effort complements recent reports on antitubulin action observed with (methoxyphenyl)-substituted methyl benzoates [13] and (trimethoxyphenyl)-substituted congeners [14] designed as prototypes of allocolchicine (**3**), as well as the description of potent new bisbenzyl and *cis*-stilbene antimitotic agents isolated from *Combretum caffrum* [15–17].

Chemistry. – MeO- and alkyl-substituted biphenyls can be obtained by aryl-aryl coupling [18], *Grignard* reaction of bromobenzenes with ethoxycyclohexenones followed by aromatization with Cu(II) [19], and ozonolysis of phenanthrenes [20]. However, for preparing the desired tetramethoxybiphenyls, we preferred to follow a route chosen by *Lespagnol* and *Schmitt* [21] *via* intermediate 5-phenylcyclohexane-1,3-diones *(cf. Scheme 1)*, and already tested with the synthesis of hartwood constituents [22], olivetol [23], and diphenic acids related to the alkaloid protostephanine [24]. Elimination of undesired phenolic OH groups introduced during the aromatization of 1,3-diones, or by a *Baeyer-*

Scheme 1



10 **11** $R^1 = R^2 = OCH_3$, $R^3 = COOCH_3$, $R_4 = H$ **16** $R^1 = R^2 = H$, $R^3 = OH$
12 $R^1 = R^2 = R^3 = R^4 = H$ **17** $R^1 = R^2 = H$, $R^3 = O$–tetrazolyl
13 $R^1 = R^2 = OCH_3$, $R^3 = R^4 = H$ **18** $R^1 = R^2 = R^3 = H$
14 $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$ **19** $R^1 = R^2 = OCH_3$, $R^3 = OH$
15 $R^1 = R^2 = OCH_3$, $R^3 = H$, $R^4 = CH_3$ **20** $R^1 = R^2 = OCH_3$, $R^3 = O$–tetrazolyl
21 $R^1 = R^2 = OCH_3$, $R^3 = H$

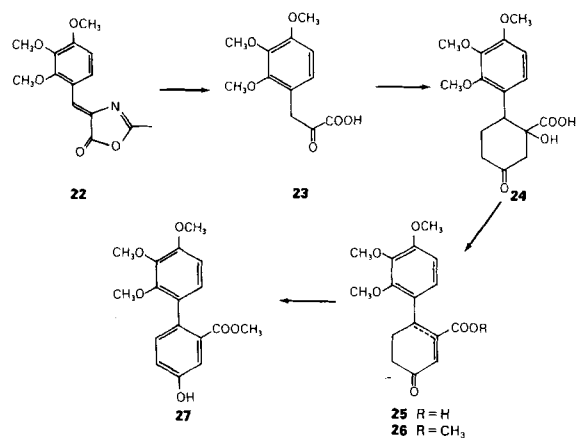
Villiger oxidation *(cf. Scheme 3)* was envisaged to be accomplishable by catalytic hydrogenation of phenyl tetrazolyl ethers and investigated earlier [25] [26].

Conversion of cyclohexane-1,3-dione into anisole, chosen to test this approach and not reported here with experimental details, showed that the route dione→enol methyl ether→resorcinol methyl ether→phenyl tetrazolyl ether→anisole could be accomplished with good overall yield. When repeated with dione **12** [21][2]) synthesis of biphenyl **18** succeeded similarly by the following reaction steps: formation of enol ether **14** from **12** by a H_2SO_4-catalyzed etherification, aromatization of **14** to **16** with Pd/black in refluxing *p*-cymene [27], formation of **17** from **16** with (chlorophenyl)tetrazole in DMF in the presence of K_2CO_3, and conversion of **17** into **18** by catalytic hydrogenation over Pd/C catalyst in AcOH at 60° [28].

Biphenyl **18** is a crystalline compound which is fully characterized, showing in particular in the ^{1}H-NMR spectrum the 8 arom. H at 7.53 (2 H), 7.34, 7.15, 7.10, 6.98 (2 H), and 6.86 ppm and the 6 H of the two MeO groups at 3.87 and 3.86 ppm. Tetramethoxybiphenyl **21** was similarly prepared by the following sequence of reactions: condensation of 2,3,4-trimethoxybenzaldehyde with acetone to ketone **10**, reaction of **10** with methyl malonate in refluxing MeOH in the presence of MeONa to keto-ester **11**, hydrolysis of **11** and decarboxylation of the resulting acid to diketone **13**. Formation of enol ether **15** from **13** was accomplished with H_2SO_4 in MeOH. Aromatization of **15** to **19** could not be achieved with Pd/black catalyst in refluxing hydrocarbons [27], or DDQ in toluene [29], and was finally accomplished, in 83.6% yield, with $Hg(OAc)_2$, a procedure used in the synthesis of olivetol [30]. Phenyltetrazolylation of **19** afforded **20** which was deoxygenated to biphenyl **21** over Pd/C catalyst in AcOH at 80°. Biphenyl **21** is fully characterized and its ^{1}H-NMR shows the 6 arom. H at 7.32, 7.10–7.07 (2 H), 7.05, 6.88, and 6.74 ppm, and the 12 H of the 4 MeO groups at 3.94, 3.91, 3.85, and 3.69 ppm.

Syntheses of tetramethoxybiphenyls **34** and **40** *(cf. Scheme 3)* were accomplished from ester **27** by classical reaction sequences. Synthesis of **27** shown in *Scheme 2* was

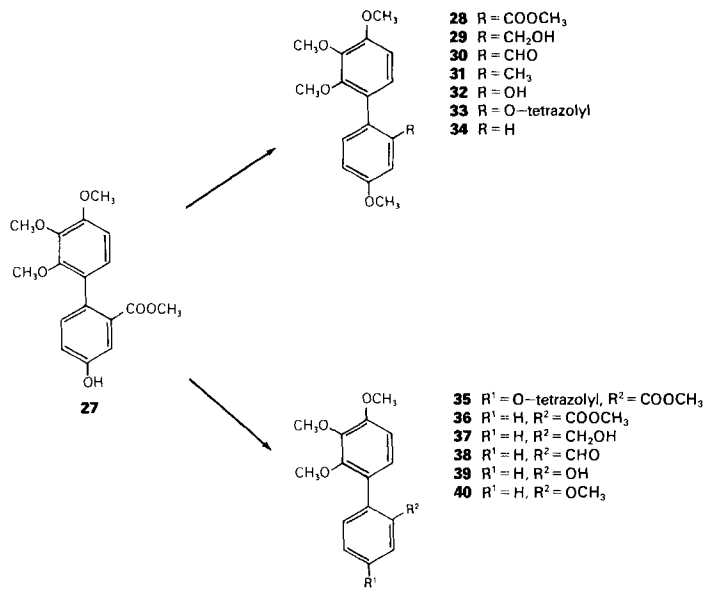
Scheme 2



[2]) We thank Dr. *Walter Dürkheimer* from the *Hoechst Co.*, Frankfurt, West Germany, for a most generous gift of differently substituted phenylcyclohexane-1,3-diones.

accomplished by a route chosen by *Umezawa et al.* for the synthesis of lycorine [31]: acid hydrolysis of oxazolone **22**, obtained from 2,3,4-trimethoxybenzaldehyde and *N*-acetylglycine in Ac_2O in the presence of AcONa, afforded pyruvic-acid derivative **23**. *Robinson* annelation of **23** with methyl vinyl ketone afforded hydroxy-acid **24**, which was converted into unsaturated acid **25** in refluxing EtOH in the presence of H_2SO_4. By esterification with MeI in acetone, acid **25** was converted without purification into methyl ester **26**. Acid **25** and ester **26** are, on the basis of their NMR spectra, contaminated with isomers containing the double bond conjugated to the aromatic ring. Aromatization of **26** to **27** was best accomplished by heating **26** over Pd/black at 200°. Ester **27** is fully characterized, showing in the ^{1}H-NMR spectrum the 5 arom. H at 7.41, 7.20, 7.01, 6.92, and 6.71 ppm, and the 4 Me signals at 3.90, 3.89, 3.67, and 3.53 ppm.

Scheme 3



With biphenyl **27** on hand, two routes were designed to accomplish the synthesis of biphenyls **34** and **40** *(Scheme 3)*. To prepare the 4′-MeO-substituted biphenyl **34**, the following reactions were carried out: methylation of **27** with MeI in acetone in the presence of K_2CO_3 afforded ether **28**, which was reduced with $LiAlH_4$ in Et_2O to alcohol **29**. Oxidation of **29** with pyridinium chlorochromate (PCC) in CH_2Cl_2 afforded a crystalline aldehyde **30**, which was converted by a *Wolff-Kishner* reduction into the crystalline biphenyl **31**. *Baeyer-Villiger* oxidation of **30** with *m*-chloroperbenzoic acid in CH_2Cl_2 afforded after hydrolysis of formate ester with 5% NaOH in MeOH phenol **32**. Phenyltetrazolyl ether **33** prepared from **32** as usual afforded after routine deoxygenation biphenyl **34**. Crystalline **34** is fully characterized and its ^{1}H-NMR spectrum shows the 6 arom. H at 7.44 (2 H), 7.03, 6.96 (2 H), and 6.74 ppm, and the 12 H of the 4 MeO groups at 3.94, 3.90, 3.86, and 3.67 ppm.

Synthesis of the C(2')-MeO-substituted biphenyl **40** was accomplished from **27** as follows. Catalytic removal of the O function was accomplished *via* **35** in the usual way to afford ester **36**. Reduction of **36** with $LiAlH_4$ in Et_2O afforded alcohol **37**, which was oxidized with PCC in CH_2Cl_2 to aldehyde **38**. *Baeyer-Villiger* oxidation of **38** afforded, after alkaline hydrolysis of the formate ester, phenol **39**, which was then converted into the desired tetramethoxybiphenyl **40** by methylation with MeI in acetone in the presence of K_2CO_3. Biphenyl **40** is a crystalline compound which shows in the ^{1}H-NMR the 6 arom. H at 7.35–7.20 (2 H), 7.02–6.95 (2 H), 6.94, and 6.72 ppm, and the 12 H of the 4 MeO groups at 3.91, 3.90, 3.79, and 3.71 ppm.

Biological Evaluation. – Compounds **21**, **28**, **29**, **31**, **34**, and **40** were first examined as potential inhibitors of the polymerization of purified tubulin *(Table, Exper. 1)*, and they were compared to colchicine (**1**), the potent *N*-acetylcolchinol *O*-methyl ether **4**, and the bicyclic tropolone compound **5**. Only two of the biphenyls, **31** and **34**, had significant inhibitory effects in this assay, the former being more potent than the latter. Compounds **1**, **4**, and **5** were all more inhibitory than the new compounds. Essentially the same result was obtained, when inhibition of the binding of radiolabelled colchicine to purified tubulin was examined *(Table, Exper. 2)*, except that **34** was somewhat more inhibitory than **31** (the relatively weak inhibition by low concentrations of non-radiolabelled colchicine (**1**) derives from the slow binding of the drug to tubulin and the use of a subsaturating amount of radiolabelled colchicine in the experiment).

The six biphenyls were examined for cytotoxicity and antimitotic effects against *L1210* murine leukemia cells *in vitro*, and compound **31** was the most active of them. It inhibited cell growth by 50% at a concentration of 0.6 μM (IC_{50} value). When cells were examined microscopically at a somewhat higher drug concentration (2 μM), multiple

Table. *Biological Properties of Biphenyls*

Agent	*Exper. 1* Tubulin polymerization[a])	*Exper. 2* Colchicine binding[b]) Inhibitor/colchicine	
		1:1	10:1
	IC_{50} [μM]	Percent inhibition	Percent inhibition
31	10–15	30	82
34	20–25	42	85
21	50–75	8	61
29	50–75	9	66
28	75–100	0	43
40	> 100	0	18
Colchicine (**1**)	4–5	24	87
N-acetylcolchinol *O*-methyl ether (**4**)	2–3	92	100
5	7.5–10	54	92

[a]) Reaction mixtures contained 1.0M monosodium glutamate (pH 6.6 with HCl), 1.0 mM $MgCl_2$, 0.4 mM GTP, 1.0 mg/ml (10M) tubulin, and various drug concentrations. All components were preincubated for 15 min at 37° prior to addition of GTP. The IC_{50} range describes concentrations at which less than and greater than 50% inhibition of polymerization after 20 min at 37° was reproducibly observed [17].

[b]) Reaction mixtures contained 0.1 mg/ml (1M) tubulin, 5M [ring-A-4-^{3}H]colchicine, and the indicated inhibitor at either 5 or 50M. Incubation was for 10 min at 37°. For further details, see [17].

mitotic figures were observed. This implies that the mechanism of action for the agent is interference with the microtubule system, consistent with its apparent binding to tubulin. (For comparison, the IC_{50} values for **1**, **4**, and **5** are 0.07, 0.009, and 0.1 μM, respectively, with mitotic arrest observed at equivalent cytotoxic drug concentrations.)

Conclusions. – Our theory that proper positioning of the 4 MeO groups in colchicinoids and colchinols (*i.e.* compounds having a six membered ring C) is important for binding to tubulin is now supported by the data presented in the *Table*. Biphenyl **34** with four MeO groups similarly placed as in colchinol ether **4** showed better inhibitory activity and did bind better to tubulin than either the *m*-MeO-substituted analog **21**, or the *o*-MeO-substituted analog **40**, the least potent of the three biphenyls. Of the three *p*-MeO-substituted analogs **28**, **29**, and **31** having an additional substituent at C(2'), compound **31** was the most potent. As a cytotoxic agent it was 1/6th as potent as *Fitzgerald*'s phenyltropolone **5** and 1/9th as potent as colchicine (**1**). The differences were still less marked between compound **31**, on the one hand, and colchicine (**1**) and compound **5**, on the other, in the *in vitro* biochemical assays in which inhibition of tubulin polymerization and the binding of radiolabeled colchicine to tubulin were examined *(Table)*.

This encourages us to speculate that hindering molecular rotation around the biphenyl axis with substituents at the C(2') and or C(6) positions may lead to agents with still greater biological activity. Synthetic and biochemical studies to examine this possibility are now in progress.

Experimental Part

General. TLC: silica gel *GHLF* plates from *Analtech;* visualization with UV light, phosphomolybdic acid, I_2, ferric chloride soln.; R_f data for $CHCl_3$/MeOH. CC: silica gel *60 (Merck)*, 230–400 mesh, 60 Å (flash chromatography). M.p.: *Fisher-Johns* melting-point apparatus. IR spectra: *Beckman IR 4230*. ^{1}H-NMR spectra: *Varian XL 300* (300 MHz). CI-MS: *Finingan 1015 D* instrument.

3-Methoxy-5-(4-methoxyphenyl)-2-cyclohexan-1-one (**14**). A mixture of *5-(4-methoxyphenyl)cyclohexane-1,3-dione* (**12**) (1 g), conc. H_2SO_4 (100 mg), and MeOH (25 ml) was maintained at r.t. for 22 h. Evaporation of MeOH gave a residue which was poured into 5% $NaHCO_3$ soln. and extracted with $CHCl_3$. The extract was washed with 5% $NaHCO_3$ soln. and H_2O, and dried ($MgSO_4$). Evaporation of solvent gave **14** (0.82 g, 77.1%) as pale yellow oil. IR ($CHCl_3$): 1640, 1605, 1200. ^{1}H-NMR ($CDCl_3$): 7.16 (*d*, J = 8.5, 2 arom. H); 6.88 (*d*, J = 8.5, 2 arom. H); 5.45 (*s*, H–C(2)); 3.80 (*s*, CH_3O); 3.73 (*s*, CH_3O); 3.30 (*m*, H–C(5)); 2.67–2.48 (*m*, 2 CH_2). MS: 233 ($M^+ + 1$).

4',5-Dimethoxy-1,1'-biphenyl-3-ol (**16**). A mixture of **14** (798 mg), Pd/black (195 mg), and *p*-cymene (3 ml) was refluxed under N_2 for 8.5 h. After cooling, Pd/black was filtered off and washed with Et_2O. The org. soln. was extracted with 5% NaOH soln. The aq. layer was acidified with conc. HCl and extracted with $CHCl_3$. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **16** (407 mg, 51.5%). Anal. sample was recrystallized from hexane. M.p. 103°. IR ($CHCl_3$): 3600, 1610, 1595. ^{1}H-NMR ($CDCl_3$): 7.49 (*d*, J = 8.7, H–C(2'), H–C(6')); 6.96 (*d*, J = 8.7, H–C(3'), H–C(5')); 6.68 (*s*, arom. H); 6.62 (*s*, arom. H); 6.37 (*s*, arom. H); 4.90 (*s*, OH); 3.85 (*s*, CH_3O); 3.83 (*s*, CH_3O). MS: 231 ($M^+ + 1$).

3,4'-Dimethoxy-5-[(1-phenyl-1H-5-tetrazolyl)oxy]-1,1'-biphenyl (**17**). A mixture of **16** (330 mg), 5-chloro-1-phenyl-1*H*-tetrazole (310 mg), and K_2CO_3 (400 mg) in DMF (2 ml) was stirred at r.t. under N_2 for 20 h. The mixture was poured into H_2O and extracted with Et_2O. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was recrystallized from MeOH to afford **17** (480 mg, 89.5%). M.p. 118°. IR ($CHCl_3$): 1610, 1590. ^{1}H-NMR ($CDCl_3$): 7.83–7.81 (*m*, 2 arom. H of *Ph*-tetrazole); 7.62–7.52 (*m*, 3 arom. H of *Ph*-tetrazole); 7.52 (*s*, arom. H); 7.50 (*s*, arom. H); 7.16 (*d*, J = 1.6, arom. H); 7.00 (*d*, J = 12.3, H–C(2'), H–C(6')); 6.93 (*d*, J = 12.3, H–C(3'), H–C(5')); 3.87 (*s*, CH_3O); 3.85 (*s*, CH_3O). MS: 375 ($M^+ + 1$).

3,4'-Dimethoxy-1,1'-biphenyl (**18**). A mixture of **17** (434 mg) and 10% Pd/C (200 mg) in AcOH (5 ml) was hydrogenated at 60° for 2.5 h. After cooling, the catalyst was filtered and washed with Et_2O. The combined filtrate and washings were washed with 5% NaOH soln. and H_2O, and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was recrystallized from hexane/(i-Pr)$_2$O to afford **18** (228 mg, 91.8%). M.p. 58–59°. IR ($CHCl_3$): 1615, 1590. ^{1}H-NMR ($CDCl_3$): 7.53 (*d*, *J* = 8.7, H–C(2'), H–C(6')); 7.34 (*t*, *J* = 7.9, H–C(5)); 7.15 (*d*, *J* = 7.9, H–C(6)); 7.10 (*d*, *J* = 1.9, H–C(2)); 6.98 (*d*, *J* = 8.7, H–C(3'), H–C(5')); 6.86 (*dd*, *J* = 7.9, 2.6, H–C(4)); 3.87 (*s*, CH_3O); 3.86 (*s*, CH_3O). MS: 214 (M^+).

4-(2,3,4-Trimethoxyphenyl)-3-buten-2-one (**10**). To a stirred mixture of 2,3,4-trimethoxybenzaldehyde (20 g), acetone (18 g), and H_2O (10 ml) was added dropwise 10% NaOH soln. (2.5 ml) at 0°, and the mixture was stirred at r.t. under N_2 for 2 h. The mixture was acidified with 3% HCl soln. and extracted with Et_2O. The org. layer was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave a yellow oil which was chromatographed. Elution with $CHCl_3$ afforded **10** (22.98 g, 95.4%) as an oil. IR ($CHCl_3$): 1670, 1640, 1620, 1595. ^{1}H-NMR ($CDCl_3$): 7.73 (*d*, *J* = 16.1, H–C(4)); 7.28 (*d*, *J* = 9.9, arom. H); 6.68 (*d*, *J* = 9.9, arom. H); 6.64 (*d*, *J* = 16.1, H–C(3)); 3.92 (*s*, CH_3O); 3.87 (*s*, CH_3O); 3.85 (*s*, CH_3O); 2.35 (*s*, $COCH_3$). MS: 237 ($M^+ + 1$).

Methyl 2,4-Dioxo-6-(2,3,4-trimethoxyphenyl)-1-cyclohexanecarboxylate (**11**, keto form). To a stirred mixture of NaOMe (9.2 g; 25% MeOH soln.) and dimethyl malonate (6.3 g) in MeOH (15 ml) was added dropwise a soln. of **10** (8.0 g) in MeOH (10 ml) at r.t. under N_2, and the mixture was refluxed under N_2 with stirring for 2 h. After cooling, MeOH was evaporated to give a residue which was partitioned between H_2O and $CHCl_3$. The aq. layer was acidified with conc. HCl and extracted with $CHCl_3$. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave **11** as an oil which was used in the next reaction without purification. Anal. sample was purified by GC. IR ($CHCl_3$): 1740, 1660, 1630, 1605. Compound **11** was a mixture of the keto form and the enol form based on ^{1}H-NMR. MS: 337 ($M^+ + 1$).

5-(2,3,4-Trimethoxyphenyl)cyclohexane-1,3-dione (**13**, keto form). A soln. of **11** in 20% NaOH (33 ml) was stirred at 120° under N_2 for 4 h. After cooling, the mixture was extracted with Et_2O. The aq. layer was acidified with conc. HCl (13 ml) and stirred at 120° under N_2 for 1 h. After cooling, the mixture was extracted with $CHCl_3$. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was recrystallized from AcOH/hexane 1:1 to afford **13** (2.77 g). The mother liquor was condensed and chromatographed in $CHCl_3$ followed by recrystallization from AcOH/hexane 1:1 to afford additional **13** (0.43 g). Overall yield from **11** was 34.0%. IR ($CHCl_3$): 1730, 1705, 1630, 1600. Compound **13** is a mixture of the keto form and the enol form based on ^{1}H-NMR spectra. MS: 278 (M^+).

3-Methoxy-5-(2,3,4-trimethoxyphenyl)-2-cyclohexen-1-one (**15**). A soln. of **13** (2.71 g) and conc. H_2SO_4 (200 mg) in MeOH (80 ml) was maintained at r.t. for 39 h. Evaporation of MeOH gave a residue which was partitioned between 5% $NaHCO_3$ soln. and $CHCl_3$. The org. layer was washed with 5% $NaHCO_3$ soln. and H_2O, and was then dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was recrystallized from Et_2O/(i-Pr)$_2$O to afford **15** (2.73 g, 96.0%). M.p. 82°. IR ($CHCl_3$): 1640, 1605. ^{1}H-NMR ($CDCl_3$): 6.85 (*d*, *J* = 8.6, arom. H); 6.64 (*d*, *J* = 8.6, arom. H); 5.44 (*s*, H–C(2)); 3.89 (*s*, CH_3O); 3.86 (*s*, CH_3O); 3.84 (*s*, CH_3O); 3.72 (*s*, CH_3O); 3.65–3.58 (*m*, H–C(5)); 2.61–2.53 (*m*, 2 CH_2). MS: 293 ($M^+ + 1$).

2',3',4',5-Tetramethoxy-1,1'-biphenyl-3-ol (**19**). A mixture of **15** (1.0 g) and $Hg(OAc)_2$ (2.0 g) in AcOH (10 ml) was refluxed for 4.5 h with stirring under N_2. The inorg. materials were filtered and washed with Et_2O. The combined filtrate and washings were extracted with 5% NaOH soln. The aq. layer was acidified with conc. HCl and extracted with Et_2O. The Et_2O extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **19** (830 mg, 83.6%) which was recrystallized from (i-Pr)$_2$O/hexane. M.p. 106°. IR ($CHCl_3$): 3600, 1610, 1595. ^{1}H-NMR ($CDCl_3$): 7.02 (*d*, *J* = 8.6, arom. H); 6.72 (*d*, *J* = 8.6, arom. H); 6.64 (*d*, *J* = 1.6, arom. H); 6.59 (*d*, *J* = 1.4, arom. H); 6.40 (*d*, *J* = 2.2, arom. H); 4.90 (*s*, OH); 3.92 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.81 (*s*, CH_3O); 3.69 (*s*, CH_3O). MS: 291 ($M^+ + 1$).

2,3,3',4-Tetramethoxy-5'-[(1-phenyl-1H-5-tetrazolyl)oxy]-1,1'-biphenyl (**20**). A mixture of **19** (830 mg), 5-chloro-1-phenyl-1*H*-tetrazole (620 mg) and K_2CO_3 (790 mg) in DMF (3.5 ml) was stirred at r.t. for 16 h under N_2. The mixture was poured into H_2O and extracted with Et_2O. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **20** (1.09 g, 87.8%) as an oil. IR ($CHCl_3$): 1625, 1610, 1600, 1540. ^{1}H-NMR ($CDCl_3$): 7.83–7.80 (*m*, 2 arom. H of *Ph*-tetrazole); 7.61–7.52 (*m*, 3 arom. H of *Ph*-tetrazole); 7.15 (*d*, *J* = 1.5, arom. H); 7.05 (*d*, *J* = 8.6, arom. H); 7.01 (*d*, *J* = 1.5, arom. H); 6.94 (*dd*, *J* = 1.5, 1.5, arom. H); 6.73 (*d*, *J* = 8.6, arom. H); 3.92 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.86 (*s*, CH_3O); 3.71 (*s*, CH_3O). MS: 435 ($M^+ + 1$).

2,3,3',4-Tetramethoxy-1,1'-biphenyl (**21**). A mixture of **20** (153 mg) and 10% Pd/C (220 mg) in AcOH (1.5 ml) was hydrogenated at 80° for 33 h. After cooling, the catalyst was filtered and washed with Et_2O. The combined filtrate and washings were washed with 5% NaOH soln. and H_2O, and dried ($MgSO_4$). Evaporation of solvent gave an oily

residue which was chromatographed. Elution with $CHCl_3$ afforded **21** (80 mg, 82.8%) as an oil. IR ($CHCl_3$): 1600, 1590, 1580. ^{1}H-NMR ($CDCl_3$): 7.32 (*dd*, *J* = 8.2, 7.6, H–C(5′)); 7.10–7.07 (*m*, H–C(2′), H–C(6′)); 7.05 (*d*, *J* = 8.6, H–C(6)); 6.88 (*dd*, *J* = 7.7, 1.7, H–C(4′)); 6.74 (*d*, *J* = 8.6, H–C(5)); 3.94 (*s*, CH_3O); 3.91 (*s*, CH_3O); 3.85 (*s*, CH_3O); 3.69 (*s*, CH_3O). MS: 275 ($M^+ + 1$).

*3-Methyl-4-[(2,3,4-trimethoxyphenyl)methylidene]-5(4*H*)-oxazolone* (**22**). A mixture of 2,3,4-trimethoxybenzaldehyde (49.8 g), *N*-acetylglycine (19.7 g), and AcONa (10.5 g) in Ac_2O (40 ml) was refluxed for 1 h with stirring. After cooling, the precipitate was collected by filtration and washed with cold H_2O and Et_2O to give **22** which was used in the next reaction without further purification. Anal. sample was recrystallized from toluene. M.p. 148°. IR ($CHCl_3$): 1790, 1760, 1650, 1580. ^{1}H-NMR ($CDCl_3$): 8.44 (*d*, *J* = 9.0, H–C(6′)); 7.56 (*s*, Ph–C*H*=); 6.77 (*d*, *J* = 9.0, H–C(5′)); 3.96 (*s*, CH_3O); 3.93 (*s*, CH_3O); 3.87 (*s*, CH_3O); 2.38 (*s*, CH_3–C(2)). MS: 278 ($M^+ + 1$).

3-(2,3,4-Trimethoxyphenyl)pyruvic Acid (**23**). A soln. of **22** and 10% HCl (75 ml) in dioxane (145 ml) was refluxed for 3 h with stirring under N_2. After cooling, dioxane was removed *in vacuo* to give a residue which was extracted with AcOEt. The org. layer was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave a residue which was recrystallized from toluene to produce **23** (12.5 g, 29.2% yield from 2,3,4-trimethoxybenzaldehyde). M.p. 134–135°. IR ($CHCl_3$): 3480, 3300–2420, 1685, 1595. ^{1}H-NMR ($CDCl_3$): 7.68 (*d*, *J* = 8.3, H–C(6′)); 7.23 (br. *s*, OH); 6.96 (*s*, CH_2); 6.76 (*d*, *J* = 8.3, H–C(5′)); 3.93 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.90 (*s*, CH_3O). MS: 255 ($M^+ + 1$).

1-Hydroxy-5-oxo-2-(2,3,4-trimethoxyphenyl)-1-cyclohexanecarboxylic Acid (**24**). To a stirred suspension of **23** (4.26 g) in 5% NaOH soln. (25 ml) was added a soln. of 3-buten-2-one (1.9 ml) in MeOH (13.5 ml) dropwise at 10°. The mixture was then stirred at r.t. for 1½ h under N_2. MeOH was removed *in vacuo*, to give a residue which was extracted with Et_2O. The aq. layer was acidified with conc. HCl and extracted with $CHCl_3$. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent followed by recrystallization from (i-Pr)$_2$O/Et_2O gave **24** (3.9 g, 71.8%). M.p. 190–191°. IR ($CHCl_3$): 3520, 3440–2440, 1720, 1595. ^{1}H-NMR ($CDCl_3$): 7.03 (*d*, *J* = 8.6, H–C(6′)); 6.64 (*d*, *J* = 8.6, H–C(5′)); 3.94 (*s*, CH_3O); 3.84 (*s*, CH_3O); 3.82 (*s*, CH_3O); 3.78 (*m*, H–C(2)); 3.03 (*d*, *J* = 14.6, 1 H, CH_2); 2.62–2.56 (*m*, 2 CH_2); 2.10–2.09 (*m*, 1 H, CH_2). MS: 289 ($M^+ - H_2O - OH$).

Mixture of Methyl 3-Oxo-6-(2,3,4-trimethoxyphenyl)-1-cyclohexene-1-carboxylate and Methyl 5-Oxo-2-(2,3,4-trimethoxyphenyl)-1-cyclohexene-1-carboxylate (**26**). A soln. of **24** (3.7 g) and conc. H_2SO_4 (0.15 ml) in 99% EtOH (115 ml) was refluxed for 4 h with stirring under N_2. After cooling, EtOH was removed *in vacuo* to give a residue which was dissolved in $CHCl_3$. The $CHCl_3$ soln. was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave **25** as an oil which was treated at the next step without further purification. A mixture of **25**, MeI (1.5 ml), and K_2CO_3 (3.0 g) in acetone (100 ml) was refluxed for 1 h with stirring under N_2. After cooling, inorg. materials were filtered and washed with $CHCl_3$. The combined filtrate and washings were condensed *in vacuo* to give a residue which was partitioned between $CHCl_3$ and H_2O. The org. layer was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **26** which was recrystallized from (i-Pr)$_2$O (2.0 g, 54.7% yield from **24**). M.p. 88°. IR ($CHCl_3$): 1730, 1685, 1620, 1600. ^{1}H-NMR ($CDCl_3$): 6.91 (*s*, olef. H); 6.61 (*d*, *J* = 8.6, arom. H); 6.55 (*d*, *J* = 8.6, arom. H); 4.51 (*d*, *J* = 4.3, CH); 3.99 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.83 (*s*, CH_3O); 3.70 (*s*, CH_3O); 2.45–2.32 (*m*, 3 H, CH_2); 2.07–2.03 (*m*, 1 H, CH_2).

The unsaturated keto ester **26** is probably a mixture with the isomer having the double bond conjugated with the Ph group. This is based on analysis of the ^{1}H-NMR spectrum, in which the signal assigned to the olefinic proton integrates to less than 1 H when compared to other 1-H resonances in the spectrum. MS: 321 ($M^+ + 1$).

Methyl 4-Hydroxy-2′,3′,4′-trimethoxy-1,1′-biphenyl-2-carboxylate (**27**). A mixture of **26** (5.12 g) and Pd/black (0.8 g) was stirred for 20 min at 200° under Ar. After cooling, $CHCl_3$ was added to this mixture, and the catalyst was filtered and washed with $CHCl_3$. The combined filtrate and washings were condensed *in vacuo* to give an oily residue which was chromatographed. Elution with $CHCl_3$ gave **27** which was recrystallized from (i-Pr)$_2$O: 2.48 g, 48.7%. M.p. 125°. IR ($CHCl_3$): 3605, 1725, 1615, 1590. ^{1}H-NMR ($CDCl_3$): 7.41 (*d*, *J* = 2.6, H–C(3)); 7.20 (*d*, *J* = 8.4, H–C(6)); 7.01 (*dd*, *J* = 2.6, 8.4, H–C(5)); 6.92 (*d*, *J* = 8.5, H–C(6′)); 6.71 (*d*, *J* = 8.5, H–C(5′)); 5.73 (*s*, OH); 3.90 (*s*, CH_3O); 3.89 (*s*, CH_3O); 3.67 (*s*, CH_3O); 3.53 (*s*, CH_3O). MS: 319 ($M^+ + 1$).

Methyl 2′,3′,4,4′-Tetramethoxy-1,1′-biphenyl-2-carboxylate (**28**). A mixture of **27** (1.8 g), MeI (1 ml), and K_2CO_3 (1.5 g) in acetone (20 ml) was refluxed for 7.5 h with stirring under N_2. After cooling, inorg. materials were filtered and washed with $CHCl_3$. The combined filtrate and washings were condensed to give a residue which was partitioned between $CHCl_3$ and H_2O. The org. layer was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave **28** which was recrystallized from (i-Pr)$_2$O: 1.69 g, 90.0%. M.p. 100°. IR ($CHCl_3$): 1725, 1600. ^{1}H-NMR ($CDCl_3$): 7.41 (*d*, *J* = 2.8, H–C(3)); 7.25 (*d*, *J* = 8.5, H–C(6)); 7.07 (*dd*, *J* = 2.8, 8.5, H–C(5)); 6.91 (*d*, *J* = 8.5, H–C(6′)); 6.71 (*d*, *J* = 8.5, H–C(5′)); 3.89 (*s*, 2 CH_3O); 3.87 (*s*, CH_3O); 3.67 (*s*, CH_3O); 3.53 (*s*, CH_3O). MS: 333 ($M^+ + 1$).

2′,3′,4,4′-Tetramethoxy-1,1′-biphenyl-2-methanol (**29**). To a stirred suspension of $LiAlH_4$ (0.15 g) in Et_2O (10 ml) was added a soln. of **28** (1.58 g) in Et_2O (90 ml) dropwise at 0°, and the mixture was stirred at r.t. for 1.5 h under N_2. To this mixture was added H_2O-saturated Et_2O dropwise and the mixture was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave **29** (1.37 g, 94.7%) as an oil. IR ($CHCl_3$): 3500, 1600. ^{1}H-NMR ($CDCl_3$): 7.14 (*d*, *J* = 8.4, H–C(6)); 7.09 (*d*, *J* = 2.6, H–C(3)); 6.90 (*dd*, *J* = 2.6, 8.4, H–C(5)); 6.87 (*d*, *J* = 8.4, H–C(6′)); 6.74 (*d*, *J* = 8.4, H–C(5′)); 4.36 (*s*, CH_2OH); 3.93 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.87 (*s*, CH_3O); 3.53 (*s*, CH_3O). MS: 304 (M^+).

2′,3′,4,4′-Tetramethoxy-1,1′-biphenyl-2-carbaldehyde (**30**). To a stirred suspension of pyridinium chlorochromate (PCC; 1.22 g) in CH_2Cl_2 (10 ml) was added a soln. of **29** (1.15 g) in CH_2Cl_2 (10 ml) in 1 portion at r.t., and the mixture was stirred at r.t. for 1.5 h. Et_2O (20 ml) was added, and the supernatant liquid was decanted from a black gum. The insoluble residue was washed with Et_2O. The combined org. soln. was passed through a short pad of *Florisil*, and the solvent was removed to give **30**, which was recrystallized from Et_2O: 1.11 g, 97.2%. M.p. 107°. IR ($CHCl_3$): 1685, 1600. ^{1}H-NMR ($CDCl_3$): 9.80 (*s*, CHO); 7.50 (*d*, *J* = 2.8, H–C(3)); 7.29 (*d*, *J* = 8.4, H–C(6)); 7.18 (*dd*, *J* = 2.8, 8.4, H–C(5)); 6.95 (*d*, *J* = 8.6, H–C(6′)); 6.76 (*d*, *J* = 8.6, H–C(5′)); 3.91 (*s*, 2 CH_3O); 3.90 (*s*, CH_3O); 3.53 (*s*, CH_3O). MS: 303 ($M^+ + 1$).

2,3,4,4′-Tretramethoxy-2′-methyl-1,1′-biphenyl (**31**). A mixture of **30** (50 mg), 80% $NH_2NH_2 \cdot H_2O$ (0.02 ml), and KOH (22 mg) in diethylene glycol (0.5 ml) was stirred for 30 min at 150°. After cooling, the mixture was poured into H_2O and extracted with Et_2O. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ followed by recrystallization from $(i\text{-}Pr)_2O$ afforded **31** (37 mg, 77.6%). M.p. 69°. IR ($CHCl_3$): 1600, 1570. ^{1}H-NMR ($CDCl_3$): 7.11 (*d*, *J* = 8.3, H–C(6′)); 6.83 (*d*, *J* = 8.4, H–C(6)); 6.81 (*d*, *J* = 2.8, H–C(3′)); 6.76 (*dd*, *J* = 2.8, 8.3, H–C(5′)); 6.70 (*d*, *J* = 8.4, H–C(5)); 3.92 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.83 (*s*, CH_3O); 3.56 (*s*, CH_3O); 2.15 (*s*, CH_3). MS: 289 ($M^+ + 1$).

2′,3′,4,4′-Tetramethoxy-1,1′-biphenyl-2-ol (**32**). A mixture of **30** (300 mg) and 3-chloroperbenzoic acid (1.5 g) in CH_2Cl_2 (20 ml) was stirred at r.t. for 4.5 h. The mixture was washed with sat. $Na_2S_2O_3$ soln. and 5% $NaHCO_3$ soln., and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was dissolved in a mixture of MeOH (2 ml) and 5% NaOH soln. (1 ml) and kept at r.t. for 1 h. Evaporation of MeOH gave a residue which was acidified with 3% HCl soln. and extracted with Et_2O. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$, followed by recrystallization from $Et_2O/(i\text{-}Pr)_2O$, gave **32** (104 mg, 36.1%). M.p. 107°. IR ($CHCl_3$): 3550, 1620, 1600, 1580. ^{1}H-NMR ($CDCl_3$): 7.19 (*d*, *J* = 8.3, H–C(6)); 7.15 (*s*, OH); 7.02 (*d*, *J* = 8.8, H–C(6′)); 6.83 (*d*, *J* = 8.8, H–C(5′)); 6.63 (*d*, *J* = 2.6, H–C(3)); 6.60 (*dd*, *J* = 2.6, 8.3, H–C(5)); 3.96 (*s*, CH_3O); 3.92 (*s*, CH_3O); 3.84 (*s*, CH_3O); 3.75 (*s*, CH_3O). MS: 291 ($M^+ + 1$).

*2,3,4,4′-Tetramethoxy-2′-[(1-phenyl-1*H*-5-tetrazolyl)oxy]-1,1′-biphenyl* (**33**). A mixture of **32** (100 mg), 5-chloro-1-phenyl-1*H*-tetrazole (75 mg), and K_2CO_3 (95 mg) in DMF (1 ml) was stirred at r.t. for 14 h under N_2. The mixture was poured into H_2O and extracted with Et_2O. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **33** which was recrystallized from MeOH: 135 mg, 90.2%. M.p. 130°. IR ($CHCl_3$): 1620, 1595. ^{1}H-NMR ($CDCl_3$): 7.54–7.39 (*m*, 5 arom. H of *Ph*-tetrazole); 7.30 (*d*, *J* = 8.5, H–C(6′)); 7.17 (*d*, *J* = 2.5, H–C(3′)); 6.93 (*dd*, *J* = 2.5, 8.5, H–C(5′)); 6.85 (*d*, *J* = 8.5, H–C(6)); 6.60 (*d*, *J* = 8.5, H–C(5)); 3.88 (*s*, CH_3O); 3.85 (*s*, CH_3O); 3.68 (*s*, CH_3O); 3.51 (*s*, CH_3O). MS: 435 ($M^+ + 1$).

2,3,4,4′-Tetramethoxy-1,1′-biphenyl (**34**). A mixture of **33** (125 mg) and 10% Pd/C (90 mg) in AcOH (1 ml) was hydrogenated at 70° for 6 h. After cooling, the catalyst was filtered and washed with Et_2O. The combined filtrate and washings were washed with 5% NaOH soln. and H_2O, and dried ($MgSO_4$). Evaporation of solvent gave **34** which was recrystallized from $(i\text{-}Pr)_2O$: 67 mg, 84.9%. M.p. 75°. IR ($CHCl_3$): 1600. ^{1}H-NMR ($CDCl_3$): 7.44 (*d*, *J* = 8.8, H–C(2′), H–C(6′)); 7.03 (*d*, *J* = 8.6, H–C(6)); 6.96 (*d*, *J* = 8.8, H–C(3′), H–C(5′)); 6.74 (*d*, *J* = 8.6, H–C(5)); 3.94 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.86 (*s*, CH_3O); 3.67 (*s*, CH_3O). MS: 275 ($M^+ + 1$).

*Methyl 2′,3′,4′-Trimethoxy-4-[(1-phenyl-1*H*-5-tetrazolyl)oxy]-1,1′-biphenyl-2-carboxylate* (**35**). A mixture of **27** (500 mg), 5-chloro-1-phenyl-1*H*-tetrazole (340 mg), and K_2CO_3 (430 mg) in DMF (3 ml) was stirred at r.t. for 18 h. Inorg. materials were filtered and washed with Et_2O. The combined filtrate and washings were washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave **35** which was recrystallized from MeOH: 666 mg, 91.7%. M.p. 119°. IR ($CHCl_3$): 1730, 1600. ^{1}H-NMR ($CDCl_3$): 7.91–7.54 (*m*, 7 arom. H); 7.43 (*d*, *J* = 8.5, H–C(6)); 6.94 (*d*, *J* = 8.6, H–C(6′)); 6.74 (*d*, *J* = 8.6, H–C(5′)); 3.91 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.70 (*s*, CH_3O); 3.57 (*s*, CH_3O). MS: 463 ($M^+ + 1$).

Methyl 2′,3′,4′-Trimethoxy-1,1′-biphenyl-2-carboxylate (**36**). A mixture of **35** (765 mg) and 10% Pd/C (500 mg) in AcOH (5 ml) was hydrogenated at 80° for 4.5 h. After cooling, the catalyst was filtered and washed with Et_2O. The combined filtrate and washings were washed with 5% $NaHCO_3$ soln. and dried ($MgSO_4$). Evaporation

of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **36** (480 mg, 96.0%) which was recrystallized from (i-Pr)$_2$O. M.p. 76°. IR ($CHCl_3$): 1720, 1600. ^{1}H-NMR ($CDCl_3$): 7.88 (*dd*, J = 1.3, 7.7, H–C(3)); 7.53 (*ddd*, J = 1.3, 7.5, 7.5, H–C(5)); 7.39 (*ddd*, J = 1.3, 7.5, 7.7, H–C(4)); 7.33 (*dd*, J = 1.3, 7.5, H–C(6)); 6.93 (*d*, J = 8.5, H–C(6′)); 6.73 (*d*, J = 8.5, H–C(5′)); 3.90 (*s*, 2 CH_3O); 3.68 (*s*, CH_3O); 3.54 (*s*, CH_3O). MS: 303 ($M^+ + 1$).

2′,3′,4′-Trimethoxy-1,1′-biphenyl-2-methanol (**37**). To a stirred suspension of $LiAlH_4$ (80 mg) in Et_2O (7 ml) was added a soln. of **36** (425 mg) in Et_2O (8 ml) dropwise at 0°, and the mixture was stirred at r.t. for 1.5 h under N_2. To this mixture was added H_2O-sat. Et_2O dropwise, and the mixture was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave **37** (363 mg, 94.1%) which was recrystallized from hexane. M.p. 85°. IR ($CHCl_3$): 3520, 3480, 1600. ^{1}H-NMR ($CDCl_3$): 7.55 (*dd*, J = 1.4, 7.4, H–C(3)); 7.44–7.34 (*m*, H–C(4), H–C(5)); 7.24 (*dd*, J = 1.6, 7.3, H–C(6)); 6.90 (*d*, J = 8.5, H–C(6′)); 6.77 (*d*, J = 8.5, H–C(5′)); 4.41 (*s*, CH_2OH); 4.39 (*s*, OH); 3.95 (*s*, CH_3O); 3.93 (*s*, CH_3O); 3.55 (*s*, CH_3O). MS: 274 (M^+).

2′,3′,4′-Trimethoxy-1,1′-biphenyl-2-carbaldehyde (**38**). To a stirred suspension of PCC (420 mg) in CH_2Cl_2 (5 ml) was added a soln. of **37** (335 mg) in CH_2Cl_2 (5 ml) in 1 portion at r.t., and the mixture was stirred at r.t. for 1.5 h. Et_2O (10 ml) was added and the supernatant liquid was decanted from a black gum. The insoluble residue was washed with Et_2O. The combined org. soln. was passed through a short pad of *Florisil*, then the solvent was removed to give **38** (305 mg, 91.7%) which was recrystallized from Et_2O. M.p. 104°. IR ($CHCl_3$): 1700, 1650, 1600. ^{1}H-NMR ($CDCl_3$): 9.86 (*s*, CHO); 8.02 (*dd*, J = 1.2, 7.8, H–C(3)); 7.64 (*dt*, J = 1.2, 7.5, H–C(5)); 7.49 (*t*, J = 7.5, H–C(4)); 7.39 (*d*, J = 7.6, H–C(6)); 7.00 (*d*, J = 8.5, H–C(6′)); 6.79 (*d*, J = 8.5, H–C(5′)); 3.94 (*s*, 2 CH_3O); 3.56 (*s*, CH_3O). MS: 273 ($M^+ + 1$).

2′,3′,4′-Tetramethoxy-1,1′-biphenyl-2-ol (**39**). A mixture of **38** (75 mg) and 3-chloroperbenzoic acid (290 mg) in CH_2Cl_2 (5 ml) was stirred at r.t. for 5 h. The mixture was washed with sat. $Na_2S_2O_3$ soln. and 5% $NaHCO_3$ soln. and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was dissolved in a mixture of MeOH (1 ml) and 5% NaOH soln. (0.15 ml), and maintained at r.t. for 1 h. Evaporation of MeOH gave a residue which was acidified with 3% HCl soln. and extracted with Et_2O. The extract was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ followed by recrystallization from (i-Pr)$_2$O gave **39** (25 mg, 34.9%). M.p. 93°. IR ($CHCl_3$): 3550, 1605. ^{1}H-NMR ($CDCl_3$): 7.29 (*m*, arom. H); 7.06–6.93 (*m*, 4 arom. H); 6.84 (*d*, J = 8.8, arom. H); 3.96 (*s*, CH_3O); 3.92 (*s*, CH_3O); 3.73 (*s*, CH_3O). MS: 261 ($M^+ + 1$).

2,2′,3,4-Tetramethoxy-1,1′-biphenyl (**40**). A mixture of **39** (23 mg), MeI (0.05 ml), and K_2CO_3 (25 ml) in acetone (1 ml) was refluxed for 7 h with stirring under N_2. After cooling, inorg. materials were filtered and washed with acetone. The combined filtrate and washings were condensed to give a residue which was partitioned between Et_2O and H_2O. The org. layer was washed with H_2O and dried ($MgSO_4$). Evaporation of solvent gave an oily residue which was chromatographed. Elution with $CHCl_3$ gave **40** (22 mg, 90.8%) which was recrystallized from (i-Pr)$_2$O. M.p. 105°. IR ($CHCl_3$): 1595. ^{1}H-NMR ($CDCl_3$): 7.35–7.20 (*m*, 2 arom. H); 7.02–6.95 (*m*, 2 arom. H); 6.94 (*d*, J = 8.5, H–C(6)); 6.72 (*d*, J = 8.5, H–C(5)); 3.91 (*s*, CH_3O); 3.90 (*s*, CH_3O); 3.79 (*s*, CH_3O); 3.71 (*s*, CH_3O). MS: 275 ($M^+ + 1$).

REFERENCES

[1] P. Dustin, 'Microtubules', Springer-Verlag, Heidelberg, 1978.

[2] S.L. Wallace, 'Textbook on Rheumatology', Eds. W.N. Kelly, E.D. Harris Jr., S. Ruddy, and C.B. Sledge, W.B. Saunders & Co., Philadelphia, 1985, pp. 871–875.

[3] H.G. Capraro, A. Brossi, 'The Alkaloids', Academic Press, Inc., New York, 1984, Vol. 23, pp. 1–70 and ref. cit. therein.

[4] A. Brossi, H.J.C. Yeh, M. Chrzanowska, J. Wolff, E. Hamel, M.C. Lin, F. Quinn, M. Suffness, J.V. Silverton, *Med. Res. Rev.* **1988,** *8,* 77.

[5] M. Rösner, H.G. Capraro, A.E. Jacobson, L. Atwell, A. Brossi, M.A. Iorio, T.H. Williams, R.H. Sik, C.F. Chignell, *J. Med. Chem.* **1981,** *24,* 257.

[6] M. Ravid, M. Gotfried, J. Bernheim, A. Brossi, 'Amyloidosis', Ed. G.G. Glenner, Plenum Publishing Co., 1988, in press.

[7] T.J. Fitzgerald, *Biochem. Pharmacol.* **1976,** *25,* 1383.

[8] M.G. Banwell, personal communication (Dept. of Org. Chemistry, University of Melbourne, Melbourne, Australia).

[9] M.H. Zweig, C.F. Chignell, *Biochem. Pharmacol.* **1973,** *22,* 2142.

[10] R. Dumont, A. Brossi, C.F. Chignell, F. Quinn, M. Suffness, *J. Med. Chem.* **1987,** *30,* 732.

[11] R. Dumont, A. Brossi, J.V. Silverton, *J. Org. Chem.* **1986,** *51,* 2515.
[12] H.J.C. Yeh, M. Chrzanowska, A. Brossi, *FEBS Lett.* **1988,** *229,* 82.
[13] a) T.R. Watson, P. Burden, E. Lacey, 8th National Convention of the Royal Australian Chemical Institute, University of New South Wales, August 24–28, 1987. Abstracts of papers L-5; b) T.R. Watson, P. Burden, E. Lacey, Boden Conference on Biomolecular Design and Development. The Australian Academy of Science. Thredbo Alpine Hotel, Kosciusko National Park, NSW, February 1–3, 1988. Abstract of paper p. 22.
[14] C.E. Powell, L. Lemon, J. Ononuju, T.J. Fitzgerald, *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987,** *46,* 1322.
[15] E. Hamel, C.M. Lin, *Biochem. Pharmacol.* **1983,** *32,* 3864.
[16] G.R. Pettit, S.B. Singh, M.L. Niven, E. Hamel, J.M. Schmidt, *J. Nat. Prod.* **1987,** *50,* 119.
[17] C.M. Lin, S.B. Singh, P.S. Chu, R.O. Dempcy, J.M. Schmidt, G.R. Pettit, E. Hamel, *Mol. Pharmacol.,* in press.
[18] F.E. Ziegler, K.W. Fowler, W.B. Rodgers, R.T. Wesler, 'Organic Synthesis', Ed. E. Vedejs, John Wiley & Sons, New York, 1987, Vol. 65, pp. 108–118, and ref. cit. therein.
[19] M. Gerecke, A. Brossi, *Helv. Chim. Acta* **1979,** *65,* 1549.
[20] T.I. Ho, C.S. Shu, M.K. Yeh, F.C. Chen, *Synthesis* **1987,** 795.
[21] A. Lespagnol, J. Schmitt, *Bull. Soc. Chim. Fr.* **1950,** 458.
[22] M. Nilson, T. Norin, *Acta Chem. Scand.* **1963,** *17,* 1157.
[23] A. Focella, S. Teitel, A. Brossi, *J. Org. Chem.* **1977,** *21,* 3456.
[24] B. Pecherer, A. Brossi, *Helv. Chim. Acta* **1966,** *49,* 2261.
[25] A. Brossi, M.F. Rahman, K.C. Rice, M. Gerecke, R. Borer, J.P. O'Brien, S. Teitel, *Heterocycles* **1977,** *7,* 277.
[26] F.L. Hsu, K.C. Rice, A. Brossi, *Heterocycles* **1982,** *12,* 2109.
[27] P. Buchs, A. Brossi, *Helv. Chim. Acta* **1981,** *64,* 681.
[28] W.J. Musliner, J.W. Gates, *J. Am. Chem. Soc.* **1966,** *88,* 4271.
[29] H.O. House, 'Modern Synthetic Reactions', 2nd edn., W.A. Benjamin, Inc., Menlo Park, California, 1972, pp. 38–44.
[30] US patent 3,729,519 assigned to *F. Hoffmann-La Roche,* Nutley, New Jersey, 1973, April 24th.
[31] B. Umezawa, O. Hoshino, S. Sawaki, H. Sashida, K. Mori, Y. Hamada, K. Kotera, Y. Iitaka, *Tetrahedron* **1984,** *40,* 1783.