Pergamon

0031-9422(94)00547-8

Phytochemistry, Vol. 38, No. 2, pp. 307 314, 1995

0031-9422/95 $9.50 + 0.00

INHIBITION OF SIGNAL-REGULATED PROTEIN KINASES BY PLANT-DERIVED HYDROLYSABLE TANNINS

G. M. POLYA,* BING HUI WANG† and L. Y. FOO‡

Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia; †Department of Chemistry, La Trobe University, Bundoora, Victoria, 3083, Australia; ‡New Zealand Institute for Industrial Research and Development, Industrial Research Ltd, Gracefield Research Centre, Gracefield Road, P.O. Box 31-310, Lower Hutt, New Zealand

(*Received in revised form* 9 *May* 1994)

Key Word Index—*Phyllanthus amarus*; Euphorbiaceae; hydrolysable tannins; protein kinase inhibitors.

Abstract—A variety of hydrolysable tannins purified from *Phyllanthus amarus* are potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalytic subunit (cAK) with IC_{50} values (concentrations for 50% inhibition) in the range 0.2–1.7 μM. The three most effective compounds of this series of hydrolysable tannins have five phenolic substituents. These three compounds are also the most effective inhibitors of wheat embryo Ca^{2+}-dependent protein kinase (CDPK), rat brain Ca^{2+}- and phospholipid-dependent protein kinase C (PKC) and Ca^{2+}-calmodulin-dependent myosin light chain kinase (MLCK). The order of sensitivity for protein kinase inhibition by the hydrolysable tannins studied is cAK > CDPK > PKC > MLCK. Thus the IC_{50} values for protein kinase inhibition by the most potent compound are 0.2 μM (for cAK), 1.8 μM (for CDPK), 26 μM (for PKC) and 56 μM (for MLCK) when protein kinase affinity is measured using synthetic peptide substrates. These hydrolysable tannin inhibitors found are the most specific and potent plant-derived inhibitors of cAK yet found.

INTRODUCTION

Plants produce a wide variety of secondary metabolites that are involved in defence against animal herbivores and microbial pathogens [1–7]. In addition, some plant secondary metabolites have been shown to have allelopathic effects enabling defence against competing plants [1, 3]. High affinity biochemical sites of action of a variety of toxic plant secondary metabolites have been demonstrated, well-known examples including inhibition of $(Na^+ + K^+)$ATPase by cardiac glycosides and inhibition of cytochrome oxidase by CN^- derived from plant cyanogenic glycosides [1, 2]. The nature of high affinity sites of action of many other plant secondary metabolites (or the metabolic products of such compounds) can be inferred in many situations when such compounds have animal hormone or pheromone properties or have evident physiological effects related to their taste or smell [1]. Nevertheless, there remains a major task of defining high affinity biochemical sites of action of a large number of plant secondary metabolites that can be reasonably presumed to interact with such sites in target microorganisms and animal herbivores.

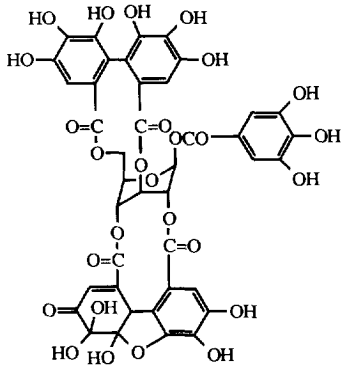
A variety of plant secondary metabolites interact with protein kinases involved in signal transduction in eukaryotes. Thus particular plant-derived anthraquinones variously inhibit animal Ca^{2+}- and phospholipid-dependent protein kinase C (PKC), Ca^{2+}-calmodulin-dependent myosin light chain kinase (MLCK) and cyclic AMP-dependent protein kinase catalytic subunit (cAK) as well as plant Ca^{2+}-dependent protein kinase (CDPK) [8]. Several plant-derived xanthones inhibit MLCK and are potent inhibitors of cAK and CDPK [9]. Particular flavonoids inhibit MLCK [10, 11], PKC [12–17], cAK [18] and tyrosine kinase [19]. Plant CDPK is relatively insensitive to inhibition by flavones suggesting that such compounds may have defensive functions by selectively inhibiting animal and fungal protein kinases [11]. The isoflavone genistein is a potent tyrosine kinase inhibitor [20] and a range of flavonol compounds, notably oligomeric procyanidins, are potent inhibitors of cAK, PKC and of plant CDPK [21]. The present paper describes the potent and selective inhibition of eukaryote signal-regulated protein kinases by a variety of hydrolysable tannins.

RESULTS

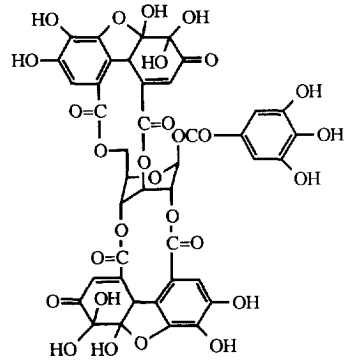
Inhibition of cAK by hydrolysable tannins

The various gallic acid derived esters (**1**–**7**) (with the exception of **3**) and gallic acid (**8**) in this study were all isolated from *Phyllanthus amarus*, a plant used in traditional medicine [22]. Compound **3** is the phenazine derivative of geraniin (**1**). Gallic acid (**8**) is 3,4,5-trihydroxybenzoic acid. All of the hydrolysable tannins tested are

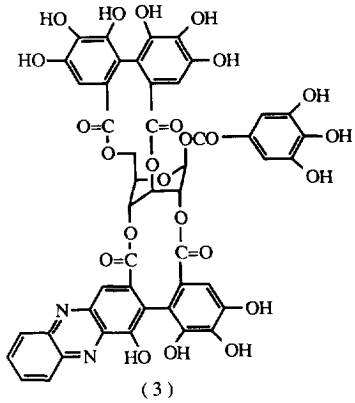
*Author to whom correspondence should be addressed.



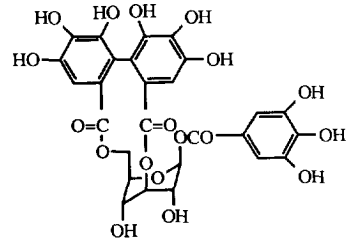
(1)



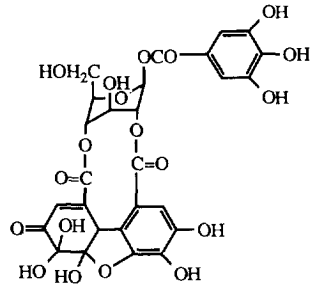
(2)



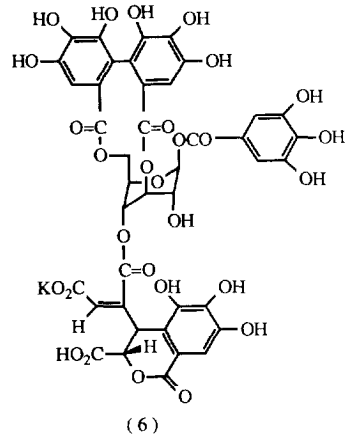
(3)



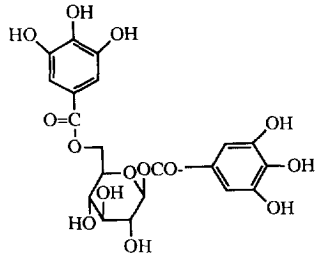
(4)



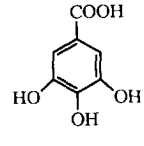
(5)



(6)



(7)



(8)

protent inhibitors of cAK with IC_{50} values in the range 0.2–2 μM (Table 1) but gallic acid (**8**) is a very poor inhibitor of the enzyme. The most potent inhibitors are compounds **1**–**3** (IC_{50} values 0.2, 0.2 and 0.4 μM, respectively; Table 1), all of which have five phenolic substituents, but compounds with less than five esterified phenolic groups have higher IC_{50} values for cAK. Thus for **6** (4 phenolic substituents), the IC_{50} is 1.0 μM; for **4** and **5** (3

Table 1. Inhibition of protein kinases by hydrolysable tannins

	IC_{50} (μM) (or % of control)					
Compound	cAK (Kemptide)	PKC (EGFRP)	MLCK (MLCP) +BSA	MLCK (MLCP) −BSA	CDPK (MLCP)	CDPK (III-S)
1	0.2	26	(63%)	56	1.8	34
2	0.2	26	152	118	4.0	45
3	0.4	28	(52%)	67	3.0	40
4	0.6	(50%)	(152%)	(75%)	26	57
5	0.6	(79%)	(93%)	(72%)	46	78
6	1.0	(81%)	(134%)	(76%)	42	53
7	1.7	(63%)	(150%)	(53%)	42	143
8	(67%)	(82%)	(205%)	(162%)	(183%)	(109%)

Protein kinases were assayed in the standard assay conditions as described in the Experimental section in the presence of increasing concentrations of inhibitors. Concentrations for 50% inhibition (IC_{50} values) were determined from interpolation from plots of protein kinase activity versus inhibitor concentration. In those instances in which compounds were relatively poor inhibitors, protein kinase activity in the presence of 167 μM inhibitor (for PKC and MLCK) or 200 μM inhibitor (for cAK and CDPK) is presented in parentheses as % of control activity (no added inhibtor). The protein substrates used for the different protein kinase assays are also given in parentheses. The final concentrations of these substrates in the various protein kinase assays were as follows: 20 μM kemptide (cAK), 3μM EGFRP (PKC), 18μM MLCP (MLCK), 20μM MLCP (CDPK) and 0.16mg ml^{-1} histone III-S (CDPK). The MLCK assays were conducted with either 0.17mg ml^{-1} BSA present (+BSA) or with no BSA present (−BSA).

phenolic groups) the IC_{50} value is 0.6 μM for both and for **7** (2 phenolic substituents) the IC_{50} value is 1.7 μM. Gallic acid (**8**) itself is ineffective (Table 1).

Lineweaver–Burk double reciprocal plots of (initial velocity)$^{-1}$ vs (substrate concentration)$^{-1}$ from enzyme kinetic data obtained in the presence or absence of **1** (Fig. 1A) indicate that **1** is a non-competitive inhibitor of cAK with respect to the synthetic peptide substrate kemptide. Thus the inclusion of **1** decreases the V_{max} but the K_m remains approximately the same (Fig. 1A). The K_i for cAK of **1** is 0.09 ± 0.08 μM (mean ± SD, from 3 determinations) noting that the IC_{50} for **1** is 0.2 μM (Table 1). However, with respect to ATP, **1** is a non-competitive inhibitor at 0.05 μM (Fig. 1B) (K_i 0.1 μM) but at 0.1 μM it is more apparently competitive with respect to ATP (Fig. 1B) (K_i 0.03 μM). At 0.2 μM, **1** is clearly competitive with respect to ATP (Fig. 1C). A similar set of observations was made with **2**. Thus, **2** is an inhibitor that is non-competitive with respect to peptide substrate (Fig. 2A), the K_i being 0.20 ± 0.03 μM (mean ± deviation from mean from 2 determinations), noting that the IC_{50} value for **2** is 0.2 μM (Table 1). However, **2** is non-competitive with respect to ATP at 0.1 μM (K_i 0.2 μM) but is apparently competitive at 0.2 μM (K_i 0.03 μM) (Fig. 2B).

Inhibition of Ca^{2+}-dependent protein kinases PKC, MLCK and plant CDPK by hydrolysable tannins

While hydrolysable tannins **1**–**7** are potent inhibitors of cAK, only **1**–**3** are good inhibitors of PKC (Table 1). Compounds **1**–**7** are all good inhibitors of plant CDPK but of these compounds **1**–**3** are the most potent inhibitors (Table 1), as found with inhibition by these compounds of both cAK and PKC. None of these compounds are good inhibitors of MLCK (Table 1) and gallic acid is not inhibitory to any of the protein kinases examined. As found with cAK, the most effective inhibitors of PKC and CDPK are **1**–**3**, the compounds with the greatest number of esterified phenolic substituents (Table 1).

The nature of the inhibition of CDPK by representative hydrolysable tannins was examined by Lineweaver–Burk kinetic analysis. As found with cAK, **1** is a non-competitive inhibitor of wheat embryo CDPK with respect to MLCP (K_i value 1.4 μM) (Fig. 3A). However, Lineweaver–Burk analysis of inhibition of CDPK by **1** indicates a mixed inhibition with respect to ATP as substrate, i.e. K_m and V_{max} are altered by inclusion of 1 or 2 μM of compound **1** (Fig. 3B).

We have previously found that while certain flavonoids (notably tricetin and quercetin) are potent inhibitors of CDPK when the synthetic peptide MLCP is used as a substrate, there is little or no inhibition by flavonoids when the histone III-S preparation is used as a substrate for the enzyme [11]. This lack of inhibition of CDPK by flavonoids in the presence of III-S histones could be due to flavonoid binding to III-S histones or histone III-S competition with flavonoids for a site on CDPK [11]. Conversely, the IC_{50} values for the interaction of the xanthones γ-mangostin and mangostin with wheat CDPK are the same with either MLCP or III-S histones as substrate [9]. Since these hydrolysable tannins are expected to bind to proteins in general [23, 24] the effect of the polypeptide substrate used on inhibition of CDPK

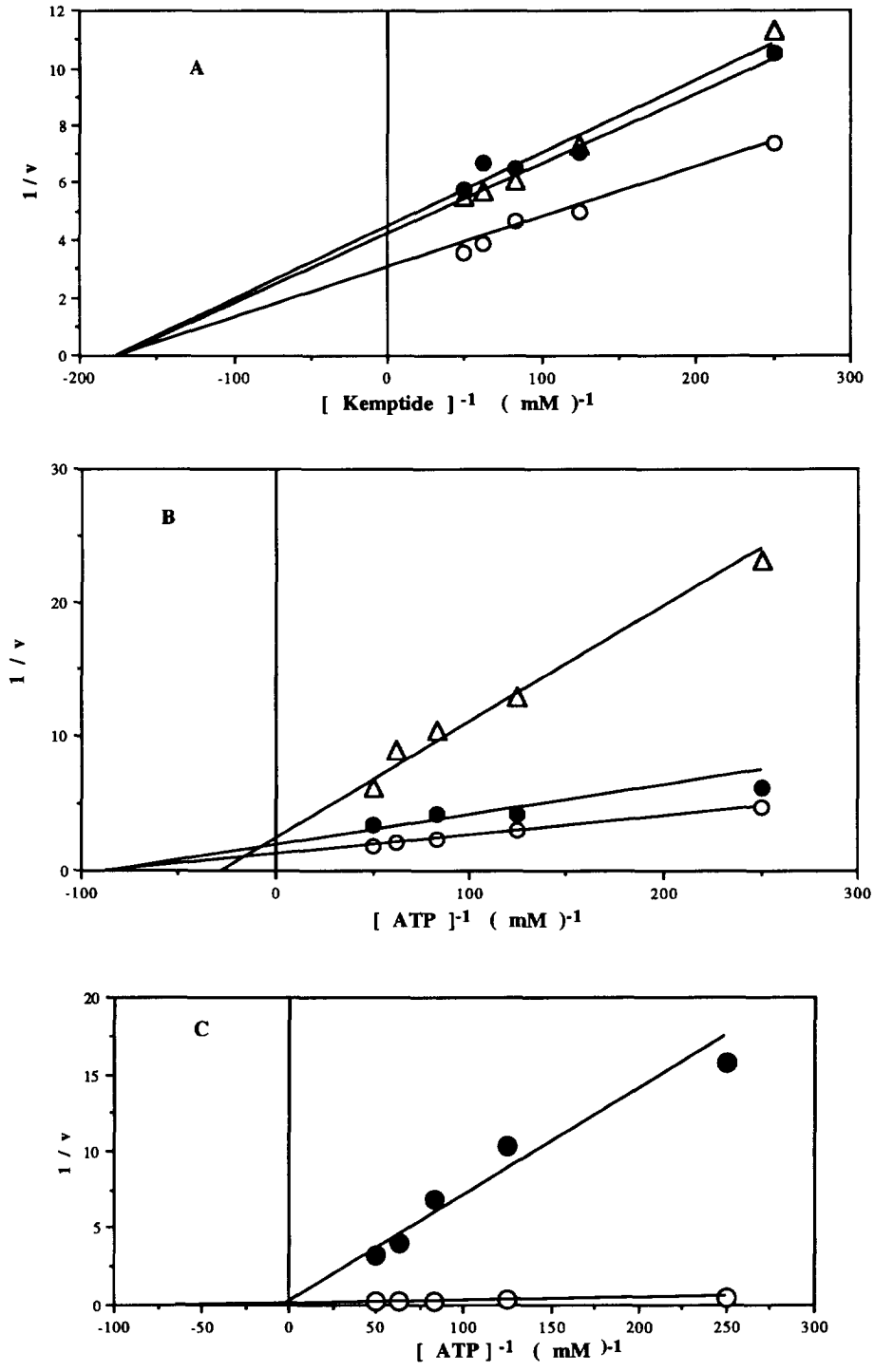


Fig. 1. Inhibition of rat liver cAK by **1**. (A) Rat liver cAK was assayed as described in the Experimental with 20 μM ATP and at various concentrations of kemptide in the absence and presence of **1**. A double-reciprocal plot of the data is presented (v^{-1} is in arbitrary units). ○—○, no added **1**; ●—●, 0.05 μM **1**; △—△, 0.1 μM **1**. (B) rat liver cAK was assayed in the standard conditions with 20 μM kemptide and at various concentrations of ATP in the presence or absence of **1**. ○—○, no added **1**; ●—●, 0.05 μM **1**; △—△, 0.1 μM **1**. (C) In a separate experiment rat liver cAK was assayed in the standard conditions with 20 μM kemptide and at various concentrations of ATP in the absence (○—○) or presence (●—●) of 0.2 μM **1**.

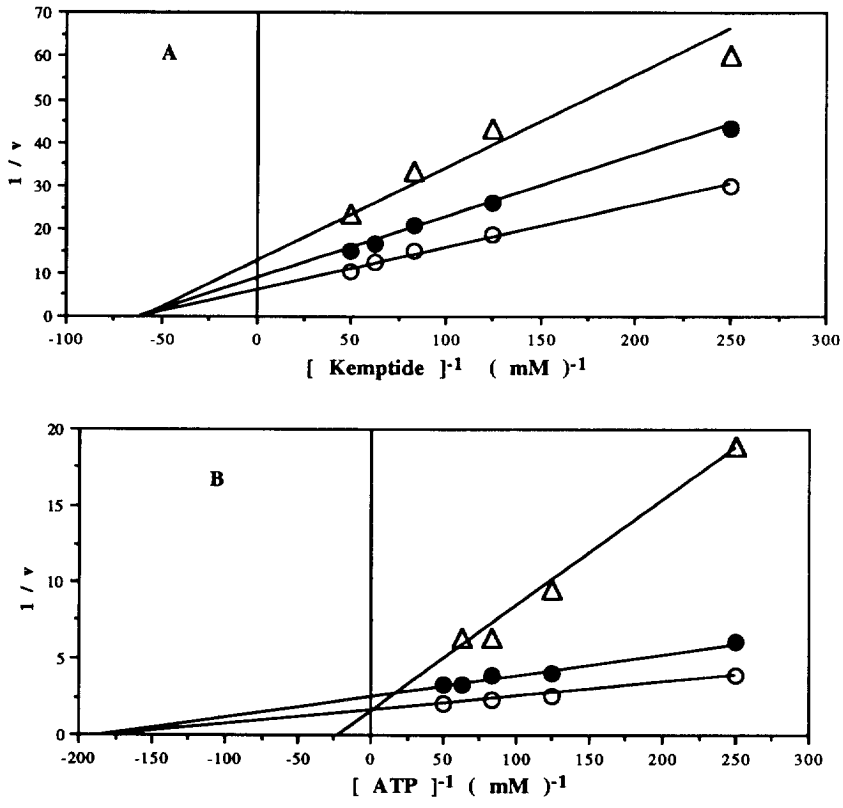


Fig. 2. Inhibition of rat liver cAK by **2**. Kinetic data were obtained and analysed as described in the legend to Fig. 1. (A) ○—○, no added **2**; ●—●, 0.1 μM **2**; △—△, 0.2 μM **2**. (B) ○– ○, no added **2**; ● –●, 0.1 μM **2**; △ △, 0.2 μM **2**.

by these compounds was examined. While **1** is a potent inhibitor of CDPK with 20 μM MLCP as substrate (IC_{50} value 1.8 μM), with 0.8 mg ml^{-1} III-S as substrate, there is only 48% inhibition by **1** at 200 μM. With 0.16 mg ml^{-1} and 0.08 mg ml^{-1} III-S as substrate the IC_{50} values for **1** are 34 and 11 μM, respectively. Bovine serum albumin (BSA) is also a substrate for wheat CDPK [25] and the IC_{50} values for **1** with 1 mg ml^{-1} BSA or 0.2 mg ml^{-1} BSA as substrate are 42 and 11 μM, respectively. Evidently BSA is less effective than the histone III-S preparation in diminishing the interaction of **1** with wheat CDPK. The presence of histone III-S also diminishes the effectiveness of the other potent inhibitors of CDPK (Table 1). Thus with 0.16 mg/ml III-S as substrate, the IC_{50} values for the potent inhibitors **1**–**3** are about 10 to 20 times greater than when the synthetic peptide MLCP is used as a substrate (Table 1). However, for the poorer inhibitors of CDPK (**4**–**7**) the IC_{50} values measured with 0.16 mg/ml III-S as substrate, are only 1.3 to 3.4 times those measured with MLCP as substrate (Table 1). The effect of increasing BSA or III-S concentration is to decrease the apparent affinity of the hydrolysable tannins for CDPK. This is consistent with either sequestration of these compounds by these proteins or competition by these proteins for the inhibitor binding site. Nevertheless, there is a very large difference between the IC_{50} values of the potent inhibitors with respect to inhibition of cAK, PKC and CDPK as determined using synthetic peptide substrates, the relative order of affinities being cAK > CDPK > PKC (Table 1). This clearly demonstrates a high specificity in these hydrolysable tannin–protein kinase interactions.

Further evidence that the compounds studied here are specific protein kinase inhibitors and are not simply acting as non-specific protein-binding entities comes from the relative lack of inhibition of MLCK by these compounds (Table 1). Deletion of BSA from the MLCK reaction mixture increases inhibition of MLCK by **1**–**7** (Table 1). However, the more effective compounds **1**–**3** are still very poor inhibitors of MLCK assayed in the absence of BSA (Table 1). Gallic acid (167 μM) does not inhibit MLCK assayed in the presence or absence of BSA (Table 1).

DISCUSSION

While hydrolysable tannins as polyphenolic compounds will interact with proteins in general [23, 24], the present results show that there is a marked specificity of the high-affinity interaction of the compounds studied here with particular eukaryote protein kinases. Thus for the most potent inhibitors (**1**–**3**), the order of affinity

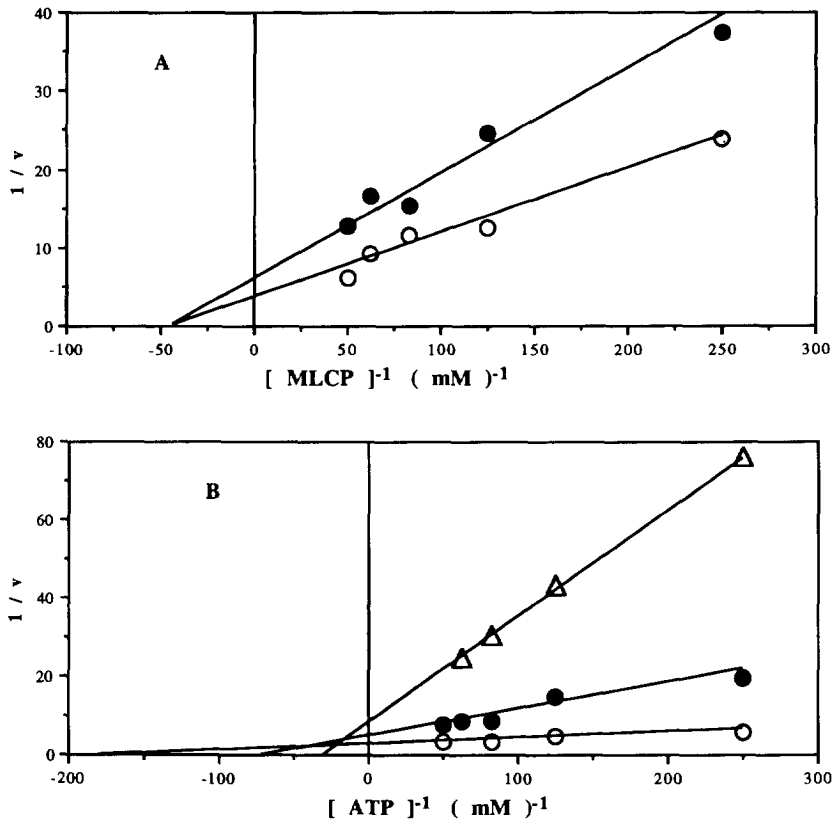


Fig. 3. Inhibition of CDPK by **1**. (A) CDPK was assayed as described in the Experimental with 20 μMATP and at various concentrations of MLCP in the absence and presence of **1**. A double-reciprocal plot of the data is presented (v^{-1} is in arbitrary units). ○—○, no added **1**; ●—●, 1 μM **1**. (B) CDPK was assayed in the standard conditions with 20 μM MLCP and at various concentrations of ATP in the presence or absence of **1**. ○—○, no added **1**; ● ●, 1 μM **1**; △ △, 2 μM **1**.

found for protein kinases assayed with synthetic oligopeptide substrates (and in the absence of added protein such as III-S or BSA) is cAK > CDPK > PKC > MLCK, the IC_{50} values for PKC inhibition being an order of magnitude greater than those for CDPK and the IC_{50} values for CDPK being an order of magnitude greater than those for cAK (Table 1). Nevertheless, the inclusion of proteins such as BSA or III-S histones in the *in vitro* protein kinase assay diminishes the apparent effectiveness of hydrolysable tannins as protein kinase inhibitors. Accordingly if these compounds do act as defensive compounds *in vivo* at the protein kinase level it is possible that such protein kinase inhibition may be attenuated by non-specific inhibitor-protein interactions.

The most potent cAK inhibitors found here are those with the greatest number of esterified phenolic substituents and are the most potent plant-derived cAK inhibitors yet described. Thus **1** and **2** have IC_{50} values of 0.2 μM for cAK (Table 1) as compared with previously reported IC_{50} values for cAK of a procyanidin tetramer of 1.4 μM [21] and of the flavonoid tricetin of 1 μM [18]. All of the more potent plant-derived cAK inhibitors yet described are polycyclic phenolics [9, 18, 21]. It is notable that cAK is much more sensitive to inhibition by hydrolysable tannins than the other eukaryote protein kinases tested (Table 1) and that cAK is inhibited by very low concentrations of a variety of other plant secondary metabolites, namely xanthones [9], anthraquinones [8], polyflavanols [21] and flavones [18] (albeit with different patterns of protein kinase specificity being observed). While Ca^{2+}-dependent protein kinases (CDPKs) are widespread in plants and there is now good evidence for plant Ca^{2+}-calmodulin-dependent kinase [26] and plant Ca^{2+}- and lipid-dependent protein kinase [27], evidence for a plant homologue of cAK is lacking. Thus, while many possible elements of a cyclic nucleotide-regulatory system have been found in plants, cyclic nucleotide-dependent protein kinases have yet to be resolved from plants [28]. Accordingly if higher plants are indeed the only eukaryotes lacking such enzymes, an effective plant defense mechanism could involve specific inhibition of the cAK of animal herbivores and fungi by plant defensive compounds. As discussed previously, plant processes could be protected from inhibition of plant CDPK by particular defensive compounds of this kind by sequestration of such compounds in hull [9] or wood [21] or, in

the case of flavonoids, by glycosylation or methylation (which greatly decreases inhibitory effectiveness) [11, 18]. Some of the hydrolysable tannins described here (**1**–**3**) have a relatively high affinity for plant CDPK although exhibiting an even greater affinity for animal cAK (Table 1). Accordingly, compartmentation of such compounds *in vivo* away from cytosolic CDPK in the tannin-producing plants could be required to prevent inhibition of CDPK-mediated signalling in such plants. The astringent taste of tannins is believed to be significant in the action of tannins in herbivore deterrence [1], tannins presumably binding to externally oriented animal cell membrane proteins involved in taste perception. It should be noted that intracellular taste transduction processes can involve second messengers and second messenger-regulated protein kinases such as cAK [29]. Endocytosis (see ref. [30]) consequent upon specific or non-specific binding of tannins to externally located membrane proteins represents a possible mechanism for tannin entry into animal and fungal cells. Very low concentrations of the more potent hydrolysable tannins are required for substantial inhibition of cAK (Table 1). Inhibition of cAK would not necessarily be lethal but would interfere with cyclic AMP-mediated regulation of signalling and metabolism [31, 32] and of signal regulated specific gene expression [33] in the target non-plant eukaryotes. The most effective hydrolysable tannin protein kinase inhibitors found here are the most potent plant-derived inhibitors of cAK yet found.

EXPERIMENTAL

Isolation of hydrolysable tannins. The isolation of the hydrolysable tannins (**1**–**7** except **3**) and the preparation of the phenazine derivative (**3**) were as previously described [22].

Protein kinase isolation and assay. Rat brain Ca^{2+}- and phospholipid-dependent protein kinase C (PKC) (sp. act. 0.6 μmol min^{-1}/mg protein with 3.5 μM EGFRP as substrate), chicken gizzard myosin light chain kinase (MLCK) (sp. act. 0.05 μmol min^{-1}/mg protein with 20 μM MLCP as substrate), rat liver cyclic AMP-dependent protein kinase catalytic subunit (cAK) (sp. act. 0.2 μmol min^{-1}/mg protein, with 20 μM kemptide as substrate) were isolated and assayed radiochemically at 30° as described previously [8, 9]. Wheat germ Ca^{2+}-dependent protein kinase (CDPK) (sp. act. 0.014 μmol min^{-1}/mg protein with 1 mg ml^{-1} histone III-S as substrate) was partially purified and assayed radiochemically at 30° in standard assay conditions as described previously [8, 9]. Test compounds were added to assays dissolved in 10% DMSO to give 2% (w/v) DMSO final concn in cAK and CDPK assays and 1.7% (w/v) DMSO final concn in PKC and MLCK assays. IC_{50} values (concns for 50% inhibition) were determined from plots of protein kinase activity versus inhibitor concn. Control protein kinase activity (no inhibitor added but with the assay containing the appropriate DMSO concn) was routinely determined in sextuplet and assays containing test inhibitors were conducted in duplicate. Standard deviations associated with control assays were routinely *ca* 10% of mean values. To avoid possible interactions of the inhibitors with relatively high concns required of protein substrates [11], synthetic peptide substrates were routinely employed in the various protein kinase assays. PKC and cAK were assayed using VRKRTLRRL-NH_2 (EGFRP) (3 μM) and LRRASLG (kemptide) (20 μM) as substrates, respectively, and CDPK and MLCK were both assayed using KKRAARATSNVFA-NH_2 (MLCP) as substrate at 20 and 18 μM, respectively.

PKC was assayed in a reaction medium (120 μl) containing 33 mM Tris (Cl^-, pH 8.0), 7 mM $MgCl_2$, 7 mM dithiothreitol, 0.2 mM EGTA, 0.7 mM $CaCl_2$, 3 μM EGFRP, 0.04 mg ml^{-1} phosphatidylserine-rich brain extract, 1.7% (w/v) DMSO, PKC and 17 μM ATP (sp. act. of [γ-^{32}P]ATP about 30 Ci mol^{-1}). The cAK assays were performed in a reaction medium (100 μl) containing 40 mM Tris (Cl^-, pH 8.0), 8 mM $MgCl_2$, 8 mM dithiothreitol, 20 μM kemptide, 2% (w/v) DMSO, cAK and 20 μM ATP (sp. act. of [γ-^{32}P]ATP *ca* 30 Ci mol^{-1}). MLCK was assayed in a reaction medium (120 μl) containing 6.4 mM Hepes (Na^+, pH 7.0), 0.8 mM Mg acetate, 0.1 mM $CaCl_2$, 0.17 mg ml^{-1} bovine serum albumin, 0.02% Tween-80, 0.16 μM calmodulin, 18 μM MLCP, 0.4 mM K-Pi, 10 mM NaCl, 0.04 mM dithiothreitol, 1.7% (w/v) DMSO, MLCK and 17 μM ATP (sp. act. of [γ-^{32}P]ATP about 30 Ci mol^{-1}). CDPK was assayed in a reaction medium (100 μl) containing 40 mM Tris (Cl^-, pH 8.0), 8 mM $MgCl_2$, 8 mM dithiothreitol 0.2 mM EGTA, 0.8 mM $CaCl_2$, 20 μM MLCP, 2% (w/v) DMSO, CDPK and 20 μM ATP (sp. act. of [γ-^{32}P]ATP about 30 Ci mol^{-1}). All assays were terminated by spotting 80 μl aliquots of reaction mixtures onto 4 cm^2 squares of phosphocellulose paper (Whatman P-81) which were subsequently washed in 500 ml 75 mM H_3PO_4 (four times) and twice in EtOH before drying and Cerenkov counting to detemine the amount of [^{32}P]phosphopeptide formed. In some instances histone III-S preparation (III-S) or bovine serum albumin (BSA) were used as protein substrates (instead of MLCP) for CDPK in which instances the same radiochemical assay procedure was employed.

Materials. Kemptide (LRRASLG), epidermal growth factor receptor-derived synthetic peptide (EGFRP; VRKRTLRRL-NH_2) and myosin light chain-based synthetic peptide (MLCP; KKRAARATSNVFA-NH_2) were obtained from Auspep, Melbourne, Australia. [γ-^{32}P]ATP (sp. act. 4000 Ci/mmol) was obtained from Bresatec, Adelaide, Australia. The histone III-S preparation and crystalline bovine serum albumin were obtained from Sigma.

Acknowledgements—This work was supported by a grant to G.M.P. from the Australian Research Council. B.H.W. was supported by a La Trobe Postgraduate Scholarship.

REFERENCES

1. Harbone, J. B. (1988) *Introduction to Ecological Biochemistry* 3rd Edn. Academic Press, London.

2. Nogrady, T. (1985) *Medicinal Chemistry. A Biochemical Approach.* Oxford University Press, Oxford.
3. Swain, T. (1977) *Ann. Rev. Plant Physiol.* **28**, 479.
4. Bell, E. A. (1980) in *Encyclopaedia of Plant Physiology* (Bell, E. A. and Charlwood, B. V., eds), Vol. 8, pp. 11–21. Springer, Berlin.
5. Bell, E. A. (1981) in *The Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds), Vol. 7, pp. 1–19. Academic Press, New York.
6. Mann, J. (1987) *Secondary Metabolism* 2nd Edn. Clarendon Press, Oxford.
7. Kúc, J. A. (1976) in *Physiological Plant Pathology, Encyclopaedia of Plant Physiology New Series* (Heitefull, R. and Williams, P. H., eds), Vol. 4, pp. 632–652. Springer, Berlin.
8. Jinsart, W., Ternai, B. and Polya, G. M. (1992) *Biol. Chem. Hoppe-Seyler* **373**, 903.
9. Jinsart, W., Ternai, B., Buddhasukh, D. and Polya, G. M. (1992) *Phytochemistry* **31**, 3711.
10. Rogers, J. C. and Williams, D. L. (1989) *Biochem. Biophys. Res. Commun.* **164**, 419.
11. Jinsart, W., Ternai, B. and Polya, G. M. (1991) *Biol. Chem. Hoppe-Seyler* **372**, 819.
12. Gschwendt, M., Horn, F., Kittstein, W. and Marks, F. (1983) *Biochem. Biophys. Res. Commun.* **117**, 444.
13. Gschwendt, M., Horn, F., Kittstein, W., Furstenberger, G., Besemfelder, E. and Marks, F. (1984) *Biochem. Biophys. Res. Commun.* **124**, 63.
14. End, D. W., Look, R. A., Shaffer, N. L., Balles, E. A. and Persico, F. J. (1987) *Res. Commun. Chem. Pathol. Pharm.* **56**, 75.
15. Picq, M., Dubois, M., Munari-Silem, Y., Prigent, A.-F. and Pacheco, H. (1989) *Life Sci.* **44**, 1563.
16. Caulfield, J. J. and Bolander, F. F. (1986) *J. Endocrinol.* **109**, 29.
17. Ferriola, P. C., Cody, V. and Middleton, E. (1989) *Biochem. Pharmacol.* **38**, 1617.
18. Jinsart, W., Ternai, B. and Polya, G. M. (1992) *Biol. Chem. Hoppe-Seyler* **373**, 205.
19. Graziani, Y., Erikson, E. and Erikson, R. L. (1983) *Eur. J. Biochem.* **135**, 583.
20. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. (1987) *J. Biol. Chem.* **262**, 5592.
21. Polya, G. M. and Foo, L. Y. (1994) *Phytochemistry* **35**, 1399.
22. Foo, L. Y. (1993) *Phytochemsitry* **33**, 487.
23. Haslam, E. (1981) in *The Biochemistry of Plants* (Conn, E. E., ed.), Vol. 7, pp. 527–556. Academic Press, New York.
24. Porter, L. J. (1989) in *Methods in Plant Biochemistry* (Dey, P. M. and Harborne, J. B., eds), Vol 1, *Plant Phenolics* (Harborne, J. B., ed.), pp. 389–419. Academic Press, New York.
25. Polya, G. M., Morrice, N. A. and Wettenhall, R. E. H. (1989) *FEBS Letters* **253**, 137.
26. Watillon, B., Kettman, R., Boxus, P. and Burny, A. (1992) *Plant Physiol.* **101**, 1381.
27. Schaller, G. E., Harmon, A. C. and Sussmann, M. R. (1992) *Biochemistry* **31**, 1721.
28. Polya, G. M., Chung, R. and Menting, J. (1991) *Plant Sci.* **79**, 37.
29. Kinnamon, S. C. and Cummings, T. A. (1992) *Ann. Rev. Physiol.* **54**, 715.
30. Mellman, I., Fuchs, R. and Helenius, A. (1986) *Ann. Rev. Biochem.* **55**, 663.
31. Cohen, P. (1989) *Ann. Rev. Biochem.* **58**, 453.
32. Cohen, P. (1992) *TIBS* **17**, 408.
33. Karin, M. and Sneal, T. (1992) *TIBS* **17**, 418.