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STUDY ON NATURAL PHENOLS WITH CATALYTIC ACTIVITY OF CYTOCHROME

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ABSTRACT

The effect of protocatechuic acid, tannic acid and trans-resveratrol on the activity of p-nitrophenol hydroxylase (PNPH), an enzymatic marker of CYP2E1, was examined in liver microsomes from acetone induced mice, trans-Resveratrol was found to be the most potent inhibitor (IC_{50} = 18.5 ± 0.4 μM) of PNPH, whi1e protocatechuic acid had no effect on the enzyme activity. Tannic acid with IC_{50} = 29.6 ± 3.3 μM showed mixed and trans-reseveratrol competitive inhibition kinetics (K_i = 1, μM and 2.1 μM, respectively). Moreover, trans-resveratrol produced a NADPH-dependent loss of PNPH activity, suggesting mechanism-based CYP2E1 inactivation. These results indicate that trans-resveratro and tannic acid may modulate cytochrome P4502E1 and influence the metabolic activation of xenobiotics mediated by this P450 isoform.

Key words: Polyphenols, Tannic acid, Protocatechuic acid, Resveratrol, CYP2El, Inhibition kinetics, Mechanism based inhibition.

INTRODUCTION

Tannic acid, protocatechuic acid and trans-resveratrol are naturally occurring phytophenols present in edible fruits and vegetables. A number of dietary polyphenols were shown to modulate the process of multistage carcinogenesis in animal models[1]. Protocatechuic acid, a simple phenolic acid, and a constituent of apples, green and black tea and herbal medicines, was reported to be an efficacious agent in reducing the carcinogenic action of nitrosoamines and related amino derivatives as well as dimethylbenz [a] anthracene in rats[2-4]. Tannic acid, a mixture of digallic acid esters of glucose, mainly present in tea, cocoa, beans, grapes, strawberries and persimmon, was shown to inhibit the mutagenicity of polycyclic aromatic hydrocarbons in Salmonella typhimurium and Chinese hamster V79 cells as well as the tumorigenicity of polycyclic aromatic hydrocarbons and N-methylnitrosourea in mouse skin, lung and forestomach[5,6]. Moreover, tannic acid applied to mouse skin caused the inhibition of covalent benzo (a) pyrene-diol-epoxide binding to epidermal DNA[7], which is considered a critical event in the initiation stage of carcinogenesis. Resveratrol (3,5,4'-trihydroxystilbene) occurs in peanuts, grapes and herbal remedies use in Japan and China[8]. It can be found in the cis and trans configurations, either free or in a glycosylated form[9,10] Burns et al., 2002). Significant concentrations (1 - 10 μM) of the a glycons cis - and trans- resveratrol are present in red wine[11]. Trans-resvecratrol has been reported to inhibit dimethy1benz [a] anthracene induced preneoplastic lesion formation in mouse[12]. The suppression of N—nitrosomethylbenzylamine (NMBA) induced esophageal turnorigenesis

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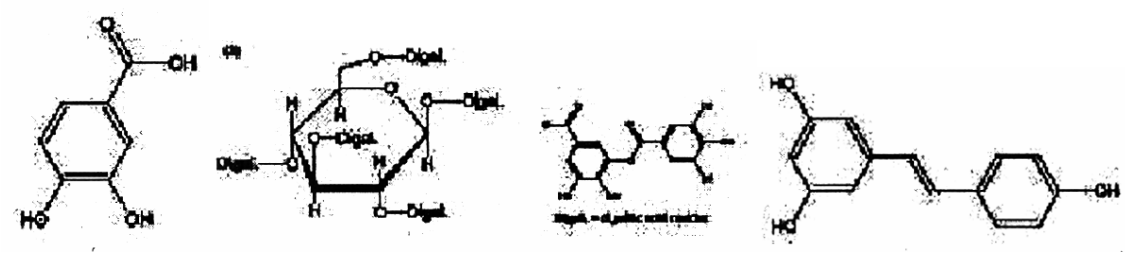
in F344 rats by resveratrol was recently reported[13]. The antimutagenic activity of resveratrol was demonstrated against a foodborne hetero-cyclic amine, 3-amino-1,4-dimethyl-5H-pyrido-[4,3-b] indole (Trp-P-1) and 2-a1ninofluorene in Salmonella bacterial tester strains[14,15].

Most chemical carcinogens require metabolic activation catalysed by cytochrome P450 in order to exert the irgenotoxic and carcinogenic effects. Thus one possible mechanism by which phenolic compounds might exert anticarcinogenic effects is through an interaction with the cytochrome P450 system, either by the inhibition or activation of certain forms of this enzyme, leading to a reduced production of the ultimate carcinogen[16-18].



(1) (2) (3)

Fig. 1: The structures of (1) protocatechuic acid, (2) tannic acid and (3) trans-resveratrol

In this paper, we have evaluated the effect of a simple phenolic acid, protocatechuic acid; a polyphenol, tannic acid and a trihydoxystilbene, trans-resveratrol (Fig. 1) on murine hepatic hydroxylation of p-nitrophenol, which represents a murine hepatic hydroxylation of p-nitro-phenol, which represents a selective substrate for cytochrome P450 2E1, CYP2E1 is vital in catalyzing the activation of nitrosodimethylamine, alkanes, halogenated hydrocarbons, and many other low molecular mass environmental chemicals[19]. Inhibition of CYP2ZE1 is expected to block the toxicity and carcinogenicity of these compounds.

EXPERIMENTAL

Materials and methods

Chemicals protocatechuic acid (purity 97%), trans-resveratrol (purity 99%), p-nitrophenol, NADP, glucose-6-phosphate and glucose phosphate dehydrogenase were purchased from Sigma (St. Louis, MO, U.S.A.). Tannic acid (purity 97%) was obtained from Aldrich (Milwaukee, WI, U.S.A.). All other compounds were commercial products of the highest purity available.

Animals, treatment and microsome preparation Female Swiss mice (7-9 weeks old, 25 g) were used in all experiments. The animals were housed in polycarbonate cages containing hardwood chip bedding at 21^{o}C and with a relative humidity of 40-70%. Commercial mouse food and distilled water were available ad libitum. The mice were treated intragastrically with 0.5 mL of 50% acetone in water for 4 consecutive days. Twenty four hours after the last treatment, the mice were killed by cervical dislocation, their livers were removed and homogenized in 0.25 M sucrose / 0.05 M Tris buffer (pH 7.5), containing 0.025 M KCl and 0.003 M $MgCl_2$. The livers from 6 animals were pooled and microsomes were prepared by centrifugation as described by Gnojkowski et al.[20] Protein concentrations were determined by the method of Lowry et al.[21]

PNPH assay. The PNPH activity was measured by p-nitrocatechol formation, according to the procedure described by Reinke & Moyer[22]. The reaction mixtures (2 mL total volume) contained acetone

induced microsomes (0.7 mg protein), p-nitrophenol (0.2 mM), an NADPH generating system (7.5 mM glucose-6-phosphate, 2 units/mL of glucose 6 phosphate dehydrogenase, 0.4 m M $NADP^+$, 5 mM $MgCl_2$), 100 mM potasium phosphate buffer (pH 6.8) and various concentrations of the tested compounds. Tannic acid was added as a water solution, whereas trans-resveratrol was dissolved in 10% Me_2SO. The final concentration of Me_2SO in the preincubation mixture did not exceed 0.1%. At this concentration Me_2SO did not affect CYP2E1 catalytic activity. Control incubations did not contain the tested compounds. The reaction was initiated by the addition of $NADP^+$ and terminated after 30 min incubation at 37°C by the addition of 0.5 mL of trichloroacetic. Samples were centrifuged for 15 min at 13000 × g and 0.1 mL of 10 Msodiumhydroxide was added to 1.5 mL of supernatant. Formation of the product (p-nitrocatechol) was measured at 526 nm, using the molar absorption coefficient of p-nitro catechol equal 9.53 $mM^{-1}cm^{-1}$. The percent inhibition of the enzyme activity by the phenolics was calculated and IC_{50} values were determined graphically, using the Microsoft Excel software program. IC_{50} values represent an average of two separate experiments.

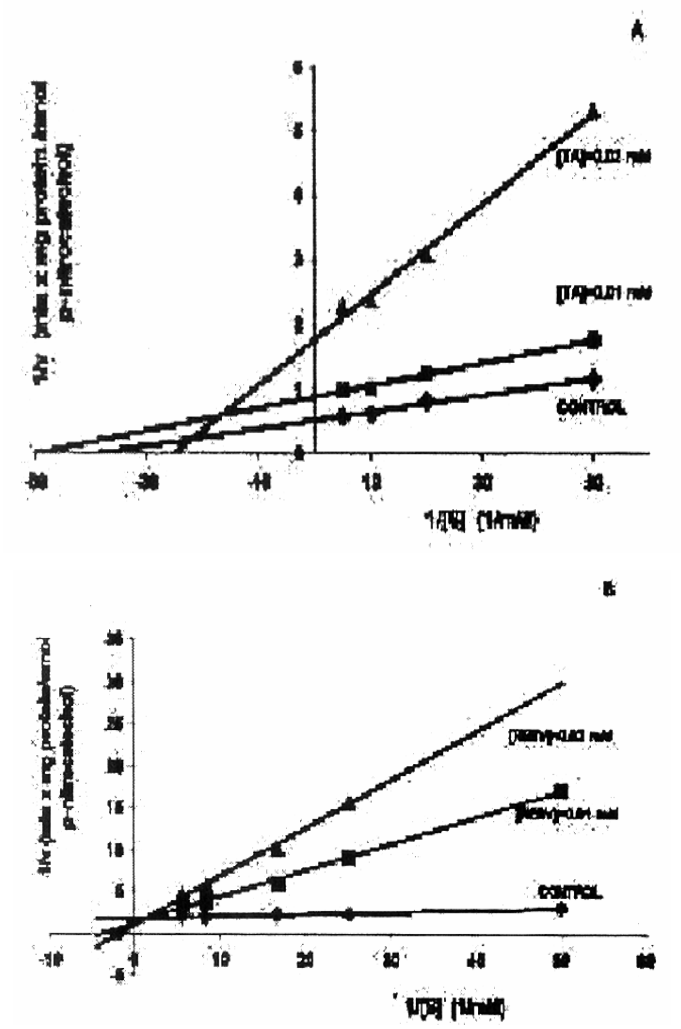


Fig. 3: Inhibition of mouse liver CYP2E1 activity by (A) tannic acid and (B) trans-resveratrol. Linswesver-Burk plots for the inhibition of p-nitrophenol hydroxylase in mouse liver microsomes. p-nitrophenol was used in the range of concentration 0.02-0.2 mM. Values are mean of triplicate determinations. Coefficient of variation did not exceed 6.6%

Inhibition kinetics

The enzyme away was as described in the preceding section. Inhibition constants (K_i) were determined for two concentrations of the tested compounds and the substrate range from 0.02 – 0.2 mM. The K_i value was determined from double reciprocal plots of the enzyme activity versus substrate concentration[23].

Inactivation of PNPH

A two stage incubation procedure was used to examine the time dependent inactivation of PNPH. trans-Resveratrol and tannic acid (at the concentrations indicated in each Fig. legend) or the appropriate vehicle (control) were preincubated (first stage incubation) with mouse liver microsomes (3.5 mg protein/mL).

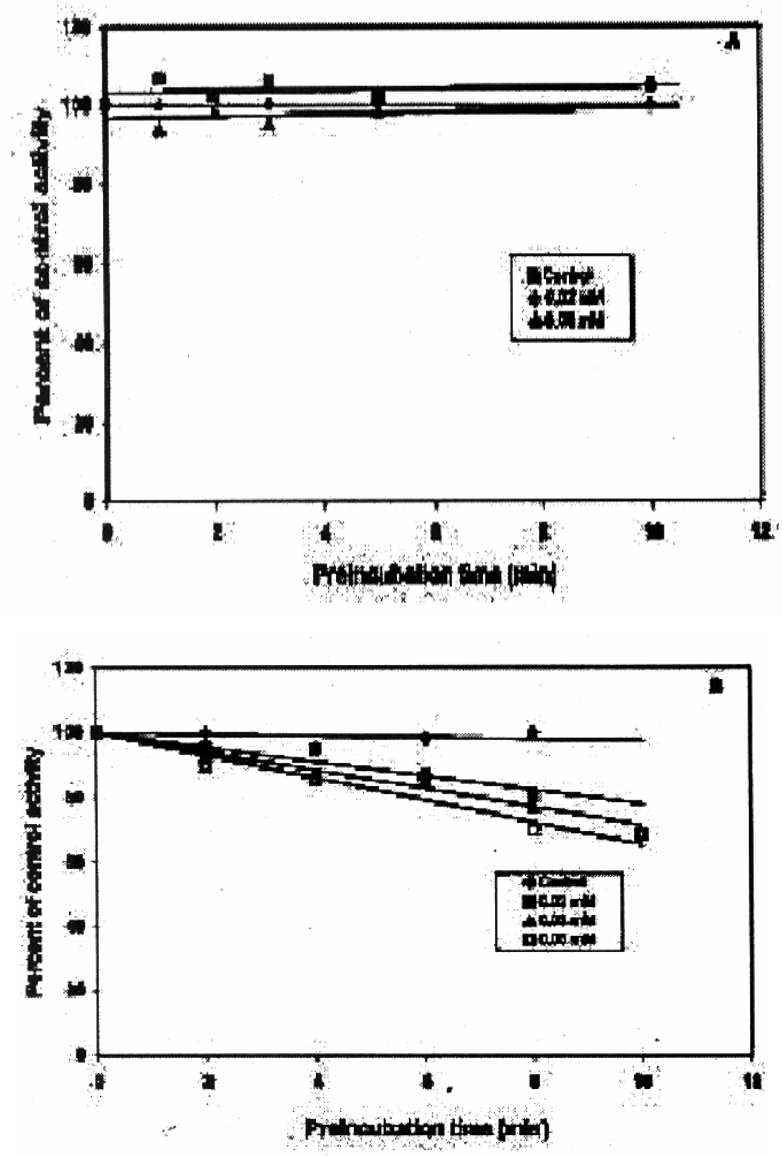


Fig. 4: Inactivation of CYP2E1 by (A) tannic acid and (B) trans-resveratrol in mouse liver microsomes. Mouse liver microsomes were preincubated with varying concentrations of inactivatiors as described under Materials and Methods. Each point represents the mean percentage of control activity of three experiments. Coefficient of variation did not exceed 9.2%

PNPH with $k_{inactivation}$ equal 0.06 min^{-1}. No inactivation of PNPH activity was detectable in absence of NADPH (data not shown). Until now, a number of compounds have been reported as selective inhibitors of cytochrome (s) P450, including both reversible and mechanism based in activators. For example, naturally occuring coumarins, found in human diet, demonstrated a structure-activity relationship for P450 selectivity and the type or mode of inhibition[24]. Among them, coriandrin was found to be a potent inhibitor and inactivator of murine and purified human P450 mi[24]. The selective inhibition of P450 2E1 by diallyl sulfide and phenethyl isothiocyanate, the components of Allium sp. and Brassicaceae vegetables, was also reported[25-27]. On the other hand, effective inhibitors of carcinogenesis are known which are not selective inhibitors of cytochromes P450. An example of this group is chlorophyllin, which non-specifically inhibits several CYP—mediated activities[28].

The phenolic compounds examined in the present study rather represent the non-selective group of P450 inhibitors. In our previous studies, tannic acid mouse hepatic cytochromes P450 1A1, 1A2 and 2B mediated enzyme activities in vitro even to a greater extent (IC_{50} 2.6-7 μM) than CYP2E1 in the current study[29,30]. As in this report, tannic acid was also a mixed inhibitor of pentoxyresorufine O-dealkylase (CYP 2B), suggesting that tannic acid may bind not only to the substrate binding site of both enzymes but also to an additional site that causes a loss of enzyme activity. Protocatechuic add, which did not show any effect on the activity of PNPH in the current study, was shown to be an efficient inhibitor of methoxy- and pentoxyresorufin O-dealkylases, the enzymatic markers of CYP1A2 and 2B, respectively[29]. In vivo, induction of CYP1A is controlled by aryl hydrocarbon receptor (AhR). trans—Resveratrol, the most potent inhibitor of PNPH in the present study, was shown to block CYP1A1 transcription in human HepG2 hepatoma cells by preventing the receptor from binding to the enhancer sequences of the CYP1A1 promoter, that regulate the transcription of the gene[31]. Moreover, of human CYP1A1 and the other AhR gene battery products, cytochromes 1B1 and 1A2 by resveratrol was also demonstrated[32,33]. Thus AhR might be a potential target for all the phytophenols examined in this paper study for modulating carcinogen activation However, the results of this study indicate that, especially for trans-resveratrol, an inhibition of P450 2E1 might be equally important. Moreover, trans-resveratrol was also reported to inhibit human recombinant CYP3A4 and CYP3A5 in vitro[34]. In our studies, trans-resveratrol was a competitive inhibitor of PNPH. This is in contrast to the observation of Piver et al.[35] who demonstrated that trans resveratrol and red wine solid components act as noncompetitive inhibitors of CYP2E1 activity in rat and human liver microsomes. In their studies, however, chlorzorazone 6-hydroxylation was used as an enzymatic marker of the catalytic activity of CYP2E1, so the difference in the mechanism of inhibition may be explained by the existence of more than one active site specific or different substrates.

Our present study also showed the ability of trans-resveratrol to inactivate cytochrome P450 2E1 in a mechanism based manner. The requirement for NADPH in the trans resveratrol inactivation of CYP2E1 indicates that it is not trans-resveratrol, but a reactive intermediate that is responsible for the inactivation of this enzyme. Mechanism based inactivation of GYP1A2 by trans-resveratrol has also been reported[36]. More detailed studies, which are underway, are necessary to explore the mechanism(s) of the inactivation of specific cytochromes P450 by this naturally occurring polyphenol. The nature of the metabolite formed in the biotransformation of trans-resveratrol catalysed by CYP2E1 remains to be identified as well. The metabolic hydroxylation of trans-resveratrol by CYP1B1 recently reported by Potter et al.[37] demonstrates that trans-resveratrol can be converted to piceatannol, a tyrosine kinase inhibitor and a compound of known anticancer activity[38].

In conclusion, our studies show that tannic acid and trans-resveratarol are potent inhibitors of CYP2E1 activity in mouse liver microsomes, but they affect this cytochrome in different ways. Future studies will focus on the mechanism of interaction and modulation of these structurally diverse polyphenols with carcinogen metabolizing enzymes.

REFERENCES

1. H. L. Newmark, Plant Ph Lies as Inhibitors of Mutations and Pnecarcinogenic Events, Can. J. Physiai. Pharmacol. Medline, **65**, 461-6 (1987).
2. M. Ohnishi, N. Yoshirni, T. Kawamori, N. Ino, Y. Hirose, T. Tanaka, I. Yamahara, H. Miyata, H. Mori, Inhibitory Effects of Dietary Protocatechuic Acid and Costunolide on 7, 12-Dimethylben[A] A11t Nmcer1e-Induceii Hamster Cheek Pouch Carcinogenesis, Jpn. J. Cancer Res. Medline, **88**, 111-9 (1997).
3. H. Mori, K. Matsunaga, Y. Tanakamaru, K. Kawabata, Y. Yamada, S. Sugie and A. Nishikawa, Effects of Protocatechuic Acid, S-Methylmetttarretlxiosulfonate or 5 -Hydroxy- 4-(2-Phenyl-(E)Etl1eny1)-2(5H)-Iinanone (KYN-54) on 4-(Methylnitrosoa1nino)-1-(3- Pyridyl}-1-Butanone-Induced Pulmonary Carcinog =·· Esis in Mice, Cancer Lett. Medline, **135**, 123-27 (1999).
4. H. Nakamura, A. Nishikawa, F. Furukawa, K. Kasahara, M. Miyauchi, H.-Y. Son and M. Hirose, Inhibihory Effects of Protncatechuic Acid on the Post-Initiation Phase of Hamster Pancreatic Carcinogenesis Induced by N-Nitmsobis (2-Oxopropyl)Amine, Anticanca Res. Medline, **20**, 3423-7 (2000).
5. C. Neplka, E. Asprodini and D. Kouretas, Tannins, Xenobiotic Metabolism and Cancer Chemoprevention in Experimental Animals, Eur. J. dn¢Gmetab Phahnaccldnet. Medline, **24**, 183-9 (1999).
6. S.-C. Chen and K.-T. Chung, Mutagenicity and Antimutagenicity Studies of Tnnnic Acid and its Related Compounds, Food Chem. Toxicol. Medline, **38**, 1-5 (2000).
7. W. Baer-Dubowska, I. Gnojkowski and W. Fenrych, Effect of Tannic Acid on Benzo [A]Pyrene-DNA Adduct Formation in Mouse Epidermis: Comparison with Synthetic Gallic Acid Esters. Nutr. Cancer, Medline, **29**, 42-7 (1997).
8. E. Ignatowicz and W. Baer-Duhowska, Resveratrol, A Natmal Chemopreventive Agent Against Degenerative Diseases, Pol. J. Phormacol. Medline, **53**, 557-69 (2001).
9. G. I. Soleas, E. P. Diamandis and D. M. Golberg, Wine as a Biological Fluid: History, Production and Role in Disease Prevention, J. Clin. Labanai. Medline, **11**, 287-313 (1997).
10. J. Bums, T. Yokota, H. Ashihara, M. E. I. Lean and A. Crozier, Plant Foods and Herbal Sources Ofresvemtrol, J. Agric. Food Chem. Medline, **50**, 3337-40 (2002).
11. G. I. Soleas, P. D. Grass Iosephy, D. M. Goldberg and P. Eleftherios, A Comparison of the Anticarcinogenic Properties of Four Wine Poly Phenols, Clin. Biochem. Medline, **35**, 119-24 (2002).
12. M. Jang, L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. Beecher, H. H. S. Fong, N. R. Farnsworth, DA, R. G. Mehta, R. C. Moon and J. M. Pezzuto, Cancer Chemopreventive Activity of Resveratrol, A Nature Product Derived from Grapes, Science Medline, **2**, **75**, 218-20 (1997).
13. Z. G. Li, T. Hong, Y. Shimada, I. Komotao, A. Kawabe, Y. Ding, J. Kaganoi, Y. Hashimoto and M. Imamura, Suppression of N-Mitrosometliylbeiazylamine (NMBA)-Induced Esophageal Tumorigenesis in F344 Rats by Resveratrol, Carcinogenesis. Medline, **23**, 1531-6 (2002).

14. F. Uenobe, S. Nakamura and M. Miyazawa, Antimutagenic Efiict of Resveratzrol against Trp-P-1, Mata! Res. Medline, **373**, 197-200 (1997).

15. I. Gusman, H. Malonne and G. Atassi, A Reammraisal of the Potential Chemoprevimtive and Chemotherapeutic Properties of Resveratrol, Carcinogenesis Medline, **22**, 1111-17 (2001).

16. F. P. Guengerich and T. Shimada, Activation of Procarcinogs by Human Cytochrome P450 Enzymes, Maia! Res. Medline, **400**, 201-213 (1998).

17. S. D. Hursting, T. J. Slaga, S. M. Fisher, J. Digiovanni and J. M. Phang, Mechanism-Based Cancer Approach ··¤ Targets, Examples, and use of Transgenic Mice, J. Nat! Cancer Inst. Medline, **91**, 215-25 (1999).

18. M. B. Sporn and N. Suh, Chemoprevention of Cancer, Carcinogenesis Medline, **21**, 575-30 (2000).

19. F. P. Guengerich, D. H. Kim and M. Lwasaki, Role of Human Cytncluome P-450 IIEI in the Oxidation of Many Low Molecular Weight Cancer Suspects, Chem. Rcs. Toxicol. Medline, **4**, 168-79 (1991).

20. J. Gnojkowski, W. Baer-Dubowska, D. Klimek and I. Chmiel, Effect of Toluidines on Drug Metabolizing Enzymes, Toxicology Medline, **32**, 335-42 (1984).

21. O. H. Lowry, N. I. Rosebrough, A. L. Farr and R. J. Randall, Protein Measurement with the Folin Phenol Reag. T. J. Biol. Chem. Medline, **193**, 230-65 (1951).

22. L. A. Reinke and N. U. Moyer, P-Nitmphenol Hydroxylatiou — A Microsomal Oxidation which is Highly Inducible by Ethanol, Dmgme Nb Dirpos. Medline, **13**, S48-52 (1985).

23. D. Voet, J. G. Voet, Rates of Enzymatic Reactions, in Biochemistry, L. Ardwin and S. Ingram Eds., Iolm Wiley S. L. Sons, Inc., New York Medline, (1995) pp. 355-60.

24. Y. Cai, D. Bennett, R. V. Nair, O. Ceska, M. I. Ashwood-Smith and I. Digiovarmi, Inhibition and Inactivation of Murine Hepatic Ethoxy- Andpentoxyresorriiiii O-Dcalkylase by Naturally Occurring Coumarins, Chem. Res. Toxicol. Medline, **6**, 872-9 (1993).

25. D. Guyonnet, C. Belloir, M. Suschetet, M.-H. Siess and A.-M. Le Bon, Liver Subcellular Fractions from Rats Treated by Organosultirr Compounds Hom Allium Modulate Mutagen Activation, Mum! Res. Medline, **466**, 17-26 (2000).

26. M. Nakajima, R. Yoshida, N. Shimada, H. Yamazaki and T. Yokoi, Inhibition and Inactivation of Human Cytochrome P450 Isoforms by Phenethyl Isothiocyanate, Drug Muah. Dilrpas. Medline, **29**, 1110-3 (2001).

27. C. S. Yang, T. I. Smith, J. -Y. Hong, Cytochrome P450 Enzymes as Targets for Ch =¢· Opreventicn against Chemical Carcinogenesis and Toxicity: Oppommities and Limitations, Cancer Rav. (Suppl). Medline, **54**, 198 2s-6s. (1994).

28. C.-H. Yun, I. W. Jhoun and F. P. Guengerich, Non-Specific Inhibition of Cytochrome P450 Activities by Chlorophyllin in Human and Rat Liver Microsomes, Carcinogenesis, Medline, **16**, 1437-40 (1995).

29. W. Baer-Dubowslca, H. Szaefer and V. Krajka-Kuzniak, Inhibition of Murine Hepatic Cytochrome P 450 Activities by Natural and Synthetic Ph =··O1ic Compounds Xenobiotica Medline, **28**, 735-43 (1998).

30. D. K. Das, M. Sato, P. S. Ray, G. Maulik, R. M. Engelman, A. A. Bertelli and A. Bertelli, Cardioprobection of Red Wine: Role of Polyphenolic Antioxidants, Drugs Exp. Clin. Ras. Medline, **25**, 115-20 (1999).

31. H. P. Ciolino, P. I. Daschner and G. C. Yeh, Retveratrol Inhibits Transcription of CYPIAI In Vitro by Preventing Activation of Aryl Hydrocarbon Receptor, Cancer Ras. Medline, **58**, 5707-12 (1998).

32. Y. J. Chun, M. Y. Kim and P. Guengerich, Resveratrol is a Selective Human Cytochrome P450 1Al Inhibitor, Biochem. Biophys. Res. Commun. Medline, **262**, 20-4 (1999).

33. T. K. H. Chang, W. B. K. Lee and H. H. Ko, Trans-Resveratrol Modulates The Catalytic Activity and Mrna Expression of the Procarcinogen-Activating Human Cytochrome P450 1Bl, Carr J. Plrysiol Pharmacol Medline, **78**, 874-81 (2000).

34. T. K. H. Chang and R. K. Y. Yeung, Eifect of Trans-Resveratrol on 7-Benzyloxy-4-Triiluorometlrylcoumariii O-Dealkylaticn Catalyzed by Human Recombinant CYP3 A4 and CYP3A5, Can. J. Physiol. Pharmacol. Medline, **79**, 220-26 (2001).

35. B. Piver, F. Berthou, Y. Dreano and D. Lucas, Inhibition of CYPSA, CYPIA and CYP2El Activities by Resveratrol and other Non Volatile Red Wine Components, Toxicol Lett. Medline, **125**, S3-91 (2001).

36. T. K. H. Chang, I. Chen and W. B. K. Lee, Dih`Erentia1 Inhibition and Inactivation of Human CYPI Enzymes by Rans-Resveratrol: Evidence for Mechanism-Based Inactivation Ot`CYP1A2, J. Pharmacol. Exp. Ther. Medline, **299**, 874-82 (2001).

37. G. A. Potter, L. H. Patterson, E. Wanoghc, P. I. Perry, P. C. Butler, T. Iliaz, K. C. Ruparelia, I1·I Lamb, P. B. Farmer, L. A. Stanley, M. D. Burke, The Cancer Preventative Agent Resveratrol is Converted to the Anticancer Agent Piceatannol by the Cytochrome P450 Enzyme CYPIBI, Br. Cancer. Medline, **86**, 774-8 (2002).

38. R. L. Geahlen and J. L. Mclaughlin, Isolation of Piceatannol as the Antileukemic Principle fiom the Seeds of Euphorbia Lagascae, Biochem. Biophys. Ros. Commun. Medline, **165**, 241-45 (1989).