Proc. West. Pharmacol. Soc. **46:** 136-138 (2003)

Synthesis and Hepatoprotective Activity of 3,4 Diacetyl; 3,4 Dibenzyl Caffeic and 4-(Dimethylamino)Cinnamic Acids

Victor Pérez-Alvarez[1*], Eduardo Fernández-Martinez[1], Martha S. Morales-Ríos[2], Rosa Amalia Bobadilla[3] and Pablo Muriel[1]

[1]Sección Externa de Farmacología, [2]Departamento de Química, CINVESTAV IPN, Apartado Postal 14-740 México 07360 D.F., México
[3]Departamento de Fisiología y Farmacología, Escuela Superior de Medicina del IPN, Plan de San Luis y Díaz Mirón, Casco de Santo Tomás México DF, CP 11340
**email: vperez@mail.cinvestav.mx*

Liver damage has been associated with deflection of arachidonic acid metabolism toward lipoxygenase products, with a simultaneous decrease of the synthesis of cytoprotective prostaglandins. Indeed leukotrienes (LT), 5-lipoxygenase products of arachidonic acid metabolism, are extensively involved in inflammatory processes.

Drugs that interfere with LT synthesis or its effects have shown hepatoprotective activity by partially preventing the rise in plasma enzyme activities after experimental acute liver damage induced by endotoxin [1], galactosamine or carbon tetrachloride (CCl_4) in rats [2].

Caffeic acid (3,4-dihydroxycinnamic acid; Fig 1), is a natural phenolic compound contained in many beverages as well as in food. It has been found to be pharmacologically active as an antioxidant, antimutagenic, anticarcinogenic agent and as a lipoxygenase inhibitor. Structurally, it is constituted by a catechol group with an α-β unsaturated carboxylic acid chain. Caffeic acid (CAF) has shown hepatoprotective properties in the CCl_4 liver damage model [3]. The deleterious effect in this model is attributed to the free radicals arising from CCl_4 metabolism.

Accordingly, CAF has shown the ability to inhibit the enzyme 5-lipoxygenase more specifically than BW755 [4]. It is also reported that caffeic acid can stimulate prostaglandin synthesis at the same concentrations at which it inhibits LT synthesis. Additionally, an antioxidant effect and free radical trapping capacity has also been described for CAF in a carcinogenesis model.

The aim of this study was to determine if substitution of catecholic group for acetyl, benziloxy or N,N dimethylamino groups in the CAF molecule could improve the hepatoprotective effect of this substance by increasing its free radical scavenger properties.

We synthesized the above mentioned derivatives (3,4 diacetyl; 3,4 dibenzyl caffeic and 4-(dimethyl-amino)cinnamic acids) and compared them with the parent compound in their ability to reduce altered hepatic markers produced by CCl_4 acute administration.

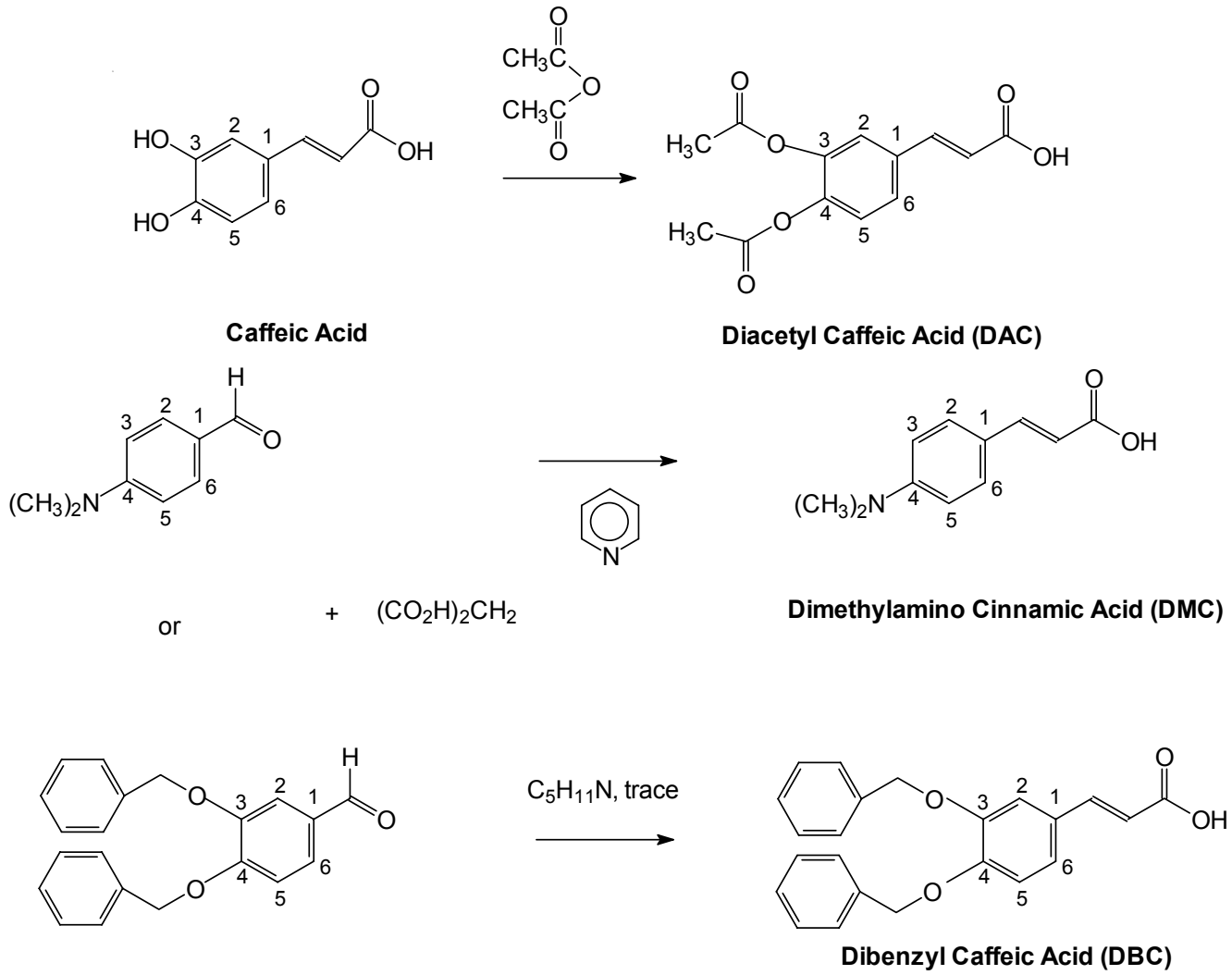
METHODS

Chemistry. Compounds were obtained as depicted in Scheme 1: briefly 3,4 diacetyl caffeic acid was obtained by reaction between caffeic acid and acetic anhydride. The condensation between 3,4 dibenzyloxy benzaldehyde and malonic acid in pyridine produced 3,4 dibenzyl caffeic acid. A similar procedure was used to obtain 4-(dimethylamino) cinnamic acid, but 4-(dimethylamino) benzaldehyde was employed. All the structures were determined using ^{1}H-NMR and ^{13}C-NMR.

Table I. Effects of experimental hepatoprotectives in the enzymatic activities of CCl_4-challenged rat serum

Treatment	Alkaline phosphatase µmol/l min	GGTP µmol/l min	ALT µmol/l min	Lipid peroxidation nmol MDA/mg protein
Control	104.69 ± 6.17 [b]	7.72 ± 0.27 [b]	19.36 ± 1.095 [b]	0.24 ± 0.05 [b]
CCl_4	200.43 ± 8.67 [a]	24.47 ± 3.19 [a]	102.71 ± 3.93 [a]	0.75 ± 0.03 [a]
Caffeic Acid	103.15 ± 13.05 [b]	16.16 ± 2.47 [a]	107.49 ± 17.71 [a]	0.49 ± 0.03 [a,b]
3,4 Diacetyl Caffeic Acid	153.97 ± 7.26 [a,b]	19.96 ± 2.99 [a]	19.22 ± 0.95 [b]	0.60 ± 0.06
3,4 Dibenzyl Caffeic Acid	117.06 ± 9.86 [b]	24.6 ± 2.14 [a]	85.92 ± 3.36 [a]	0.17 ± 0.06 [b]
4-(Dimethylamino) Cinnamic Acid	154.16 ± 12.35 [a,b]	6.74 ± 0.53 [b]	48.64 ± 4.75 [a,b]	0.74 ± 0.07 [a]

The values are the means of separate determinations in duplicate assays for 8 different animals ± SEM.
[a] $p < 0.05$: significantly different from control values.
[b] $p < 0.05$: significantly different from CCl_4-treated group.
MDA = Malondialdehyde.



Scheme 1. Synthetic route of non-phenolic Caffeic acid derivatives

Pharmacology. 48 male Wistar rats weighing between 200 and 250 g fed *ad libitum* a Purina Chow diet were used in these experiments. Acute liver damage was produced by CCl_4 administration (4 g/kg) dissolved in olive oil (1:1).

Rats were divided into 6 groups. Group 1 received a single dose of olive oil; the remaining groups received 0.4 g /100g CCl_4 dissolved in olive oil (1:1). While group 2 got no additional treatment, groups 3-6 received caffeic acid or derivatives (3 x 50 mg/kg) 12 hr before, at the time and 12 hr after CCl_4 administration. All treatments were given by means of an intragastric tube. The derivatives administered were: caffeic acid (group 3), 3,4 diacetyl caffeic acid (group 4), 3,4 dibenzyl caffeic acid (group 5) and 4-(dimethylamino) cinnamic acid (group 6). Rats were anesthetized with diethyl ether 24 hr after CCl_4 administration. Blood was obtained by heart puncture, and the liver was excised. Serum was used for determination of gamma-glutamyl transpeptidase (GGTP) [5], alkaline phosphatase (AP) [6], and alanine aminotransferase (ALT) [7] activities. Pieces of the liver were handled for lipid peroxidation products determination [8].

For statistical analysis, one way ANOVA with Dunnett and Student Newman Keuls test were performed to compare groups. In all cases a difference was considered significant when $p < 0.05$.

RESULTS: The presence of acute liver damage in the group treated with CCl_4 alone was evidenced by significative ($p < 0.05$) increases in the serum

activities of alkaline phosphatase, alanine aminotransferase and on the malondialdehyde hepatic levels. When CCl_4-treated rats were compared with those which received CCl_4 plus the lipoxygenase inhibitor caffeic acid (Table I), the CCl_4 - induced rise in alkaline phosphatase serum activity was prevented but had no significant effect on the activity of alanine aminotransferase, although there was a significant protection against the production of malondialdehyde (main product of lipid peroxidation).

Diacetyl caffeic acid improved hepatotoxicity markers only marginally. Alkaline phosphatase activity was only partially restored while GGTP and lipid peroxidation markers were little affected. ALT was, however, returned to control levels. Dibenzyl caffeic acid, like caffeic acid, returned alkaline phosphatase to near normal but was unable to prevent the increase in alanine aminotransferase activity and yet had a significant beneficial effect on lipid peroxidation. Dimethylamino caffeic acid, on the other hand, partially prevented alkaline phosphatase activity while completely preventing the rise in GGTP. The dimethylamino derivative had no effect on lipid peroxidation measures and only partially reversed the effect of CCl_4 on ALT.

CONCLUSIONS: The aim of this work was to determine if the substitution of a catecholic group for acetyl, benziloxy or N,N dimethylamino groups could improve the hepatoprotective properties of caffeic acid *in vivo*.

1. We successfully synthesized three non-phenolic analogs of cinnamic acid.
2. Acute liver damage was effectively achieved by CCl_4 administration and as we had previously reported [3], CAF can partially inhibit the biochemical markers of this effect.
3. Diacetyl caffeic acid did not produce a significant hepatoprotection suggesting that the acetyl group decreased the CAF's scavenger potential or dropped the affinity of this catechol for 5-lipoxygenase enzyme when hydrogen atoms were replaced for acetyl groups.
4. The *p*-amino substituted and no catecholic analog dimethylamino cinnamic acid, did not show a clear pattern, since it improved significantly GGTP but only partially improved Alkaline Phosphatase and ALT.
5. The more interesting compound turned out to be dibenzil caffeic acid because it improved the same markers that CAF did. This agrees with our initial propose that benzylic methylens could stabilize the molecule and increase or keep free radical scavenger properties.

ACKNOWLEDGEMENTS: The technical assistance of QFI Isabel Wens and Mr. Antonio Sanchez Trujillo (Sección de Farmacología CINVESTAV-IPN) is gratefully acknowledged. This work was supported in part by the grant 38503-M, Conacyt (Mexico).

REFERENCES

1. Keppler D, Hagman W, Rapp S, Denzaigner C: Hepatol 5: 883 (1985).
2. Pérez-Alvarez V, Bobadilla-Lugo RA, Muriel P, Favari L and Villanueva-Lopez C: Pharmacol 47: 330 (1993).
3. Reyes MT, Mourelle M, Hong E, Muriel M: Drug Devel Res 36:125 (1995).
4. Koshihara Y, Neichi T, Murota S, Lao A, Fujimoto Y and Tatsuno T: Biochim Biophys Acta 792:92 (1984).
5. Glossman M and Neville DM: FEBS letters 19:340 (1972).
6. Bergmeyer HV, Grabl M and Walter HE: Enzymes (ed) HV Bergmeyer, Methods in enzymatics analysis, Verlag-Chemie, Weinhein, p. 267 (1983).
7. Reitman S and Frankel S: Am J Clin Pathol 28:56 (1957).
8. Buege JA and Aust SD: Methods Enzymol 53:302 (1978).