Journal of Natural Products
Vol. 53, No. 4, pp. 989-992, Jul-Aug 1990

ANTIVIRAL ACTIVITY OF TERNATIN AND MELITERNATIN, 3-METHOXYFLAVONES FROM SPECIES OF RUTACEAE[1]

C.M.O. SIMÕES,*[,2]

Laboratory of Pharmacognosy, Department of Pharmaceutical Sciences, University of Santa Catarina, Florianópolis, Brazil

M. AMOROS, L. GIRRE,

Laboratory of Pharmacognosy and Mycology, Faculty of Pharmacy, University of Rennes I, Rennes, France

J. GLEYE,

Laboratory of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Toulouse III, Toulouse, France

and M.-TH. FAUVEL

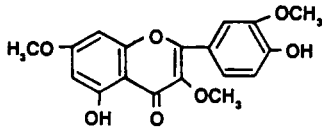
Institute for the International Map of the Vegetation, Toulouse, France

ABSTRACT.—Two 3-methoxyflavones isolated from the Rutaceae, ternatin [**3**] and meliternatin [**4**], were tested against DNA and RNA viruses. Under the same experimental conditions, only ternatin inhibited the cytopathic effect caused by all the tested viruses and reduced the viruses infectivity on the multistep virus replication. Ternatin was more effective against the naked viruses tested, especially poliovirus, and it is also active against the DNA viruses tested, especially adenovirus. Structure-activity relationships are discussed

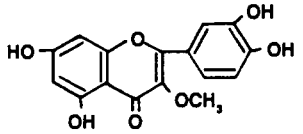
The triterpenoid saponins (1–3) and some flavonoids (4,5) were selected by our laboratory for investigation of their potential as antiviral drugs.

Naturally occurring flavonoids with antiviral activity have been recognized for nearly 40 years (6), and many investigations have been published on this subject. The most interesting results were found for an anticoxsackievirus and antirhinovirus flavone **1** (Ro-09-0179) (7), an antirhinovirus chalcone (Ro-09-0410) (8,9), an antirhinovirus 4′-6-dichloroflavan (BW 683 C) (8,10), and also for the highly active antipoliovirus 3-methoxyflavones. The elucidation of the mechanism of action of 3-methylquercetin [**2**], belonging to the latter group, was also investigated (11–13).

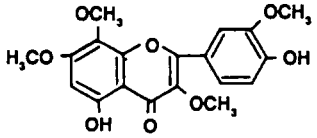
The encouraging results of the latter group prompted us to study the effects of two other different 3-methoxyflavones: ternatin [**3**] (5,4′-dihydroxy-3,7,8,3′-tetramethoxyflavone) isolated from *Evodia madagascariensis* Baker (Rutaceae)



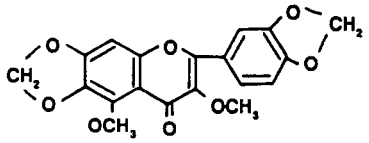
1



2



3



4

[1]Presented at the "Brazilian-Sino Symposium on Chemistry and Pharmacology of Natural Products," Rio de Janeiro, Brazil, December 1989.

[2]Present address: Laboratory of Pharmacognosy, Faculty of Pharmacy, Avenue Léon-Bernard, F-35043 Rennes, France.

TABLE 1. Antiviral Activity of Ternatin [3] Isolated from *Evodia madagascariensis* on Vero Cells.

MOI[a] ($TCID_{50}$/Cell)	Virus														
	Adenovirus			HSV-1			HSV-2			VSV			Poliovirus		
	ED_{50}[b]	VR[c]	TI[d]	ED_{50}	VR	TI	ED_{50}	VR	TI	ED_{50}	VR	TI	ED_{50}	VR	TI
10^{-2}	10 (3.74 μg/ml)	1.5	20.0	—	0	—	100 (37.4 μg/ml)	0.6	2.0	48 (17.95 μg/ml)	0.9	4.2	4 (1.5 μg/ml)	1.9	50.0
10^{-3}	10 (3.74 μg/ml)	1.7	20.0	44 (16.46 μg/ml)	0.8	4.6	14 (5.23 μg/ml)	1.5	14.4	38 (14.21 μg/ml)	1.2	5.2	3 (1.12 μg/ml)	2.0	66.6

[a]MOI = Multiplicity of infection.

[b]ED_{50} = Effective drug concentration required to inhibit by 50% the different viruses, cytopathic effect (CPE) (μM); (−) no antiviral action.

[c]VR = The sum of the values assigned to the CPE of all treated/infected cells, subtracted from the sum of the values of the CPE in equal number of virus control, divided by ten times the number of test wells used per each drug concentration.

[d]TI = Therapeutic index in vitro, calculated by dividing the drug CyD_{50} (200 μM for **3**) by its ED_{50}.

(14) and meliternatin [**4**] (3,5-dimethoxy-6,7:3′,4′-dimethylenedioxyflavone) isolated from *Melicope indica* Wight (Rutaceae) (15).

After 96 h of treatment, concentrations of 200 μM for ternatin and 800 μM for meliternatin did not inhibit cell growth, whereas higher concentrations induced granularity, retraction, and then lysis of Vero cells. For the determination of antiviral activity, we have used concentrations ranging from 5 to 100 μM for ternatin and 100 to 700 μM for meliternatin. Under the same experimental conditions, only ternatin reduced the cytopathic effect (CPE) caused by all the tested viruses. No activity was found against these viruses for meliternatin. Results are shown in Table 1.

According to Sidwell and Huffman (20), a virus-rating (VR) of >1.0 is indicative of definite antiviral activity, whereas a VR of 0.5 to 0.9 indicates moderate activity. Our results demonstrated that at a multiplicity of infection (MOI) of 10^{-2} and 10^{-3} 50% tissue culture infective dose ($TCID_{50}$)/cell, the VR values of ternatin ranged from 0.6 to 2.0, which would indicate that this flavonoid presents antiviral activity against the tested viruses. The in vitro therapeutic indexes (TI), depending on the MOI and the different viruses, could be considered acceptable (21), because one of the essential requirements for a prospective antiviral agent is its high therapeutic index.

Under a multistep virus replication test, ternatin reduced the viral titers of poliovirus, adenovirus, HSV-2, VSV, and HSV-1 by 5, 3, 2, 2, and 1 $\log_{10}$, respectively. The results showed that ternatin was more effective against the naked viruses tested (adenovirus-DNA group) and especially against poliovirus (RNA group), in accordance with the remarks made by Vanden Berghe *et al.* (16), who demonstrated that 3-methoxyflavones are highly active against picornaviruses but not against DNA viruses. We, moreover, found that ternatin also inhibited the CPE and reduced the viral titers of the DNA viruses tested, especially adenovirus.

We have noticed analogous results for 3-methylquercetin [**2**] and ternatin [**3**]: at a concentration of 5 μg/ml compound **2** drastically inhibited the viral titer of poliovirus (11), whereas compound **3**, at 1.12 μg/ml, reduced the viral titer of this same virus by 5 $\log_{10}$.

Preliminary studies on structure-activity relationships of flavonoids indicated that only the aglycone seemed to be required, because all tested glycosides were inactive. The presence of the 3-methoxy group, the carbonyl function, and the C-2–C-3 double bond are also necessary for antiviral action. Methylation of free hydroxyl groups, especially in the C-5 and C-4′ positions, seems to decrease the antiviral potency (22).

Our results confirm these statements and also allow us to state that when methylenedioxy groups are introduced at C-6–C-7 and C-3′–C-4, the antiviral activity disappears, even if the C-3 methoxy group is maintained. Moreover, the anti-DNA virus activity detected only for ternatin seems to be related to its C-8 methoxy group, because other tested 3-methoxyflavones (**4**, **2**, and Ro-09-0179) without this function lacked anti-DNA virus activity (7,11).

EXPERIMENTAL

DRUGS.—Solutions of each flavonoid were prepared in MeOH- 3% MeOH (1:1), which is a nontoxic solvent concentration for the cells (16,17). They were stored at −20° until used and then diluted in the cell culture medium for the tests. All samples were filtered in a Millex-GS-0.22-μm filter under aseptic conditions.

CELLS AND VIRUSES.—Vero cells were cultivated at 37° in Eagle's minimal essential medium (MEM) with Earle's saline, supplemented with 10% inactivated fetal calf serum and antibiotics. HSV-1 and HSV-2 were isolated at Pontchaillou Hospital, Rennes; adenovirus type 2 came from the Institute of Virology, Strasbourg; poliovirus type 2 was propagated in our laboratory; and VSV was supplied by the Virology Department, Rennes. Virus stocks were prepared by inoculating Vero monolayers at low MOI and incubating them at 37°. Two days after infection, the cul-

tures were frozen and thawed three times and the preparations were clarified by centrifugation at low speed to remove cell debris. The resulting supernatant fluids were maintained at −70°. Virus titrations were performed by the Reed and Muench dilution method (18). The viral titers were estimated from cytopathogenicity and expressed as 50% tissue culture infective dose per milliliter ($TCID_{50}$/ml): 2×10^6 for HSV-1 and VSV and 2×10^7 for HSV-2, adenovirus, and poliovirus.

CYTOTOXICITY TEST.—The method used was essentially the one described by Vanden Berghe *et al.* (19). The CyD_{50} values are defined as the maximum drug concentration which causes cytotoxic effects in 50% of the cultured cells, and values lower than those obtained in these tests were then used in antiviral tests.

DETERMINATION OF ANTIVIRAL ACTIVITY.—It was detected by measuring the inhibition of CPE with estimation of ED_{50}, VR, and TI (1) and by reduction of infectious titers in a multistep virus replication (11).

ACKNOWLEDGMENTS

One of us (C.M.O.S.) is indebted to the CNPq (Brazil) for the award of her doctoral fellowship. We thank Dr. Françoise Sauvager for helpful discussions and Mrs. Baril, Mrs. Fromont, and Mrs. Garel for their excellent technical assistance. The financial help from Langlois Foundation is gratefully acknowledged.

LITERATURE CITED

1. M. Amoros, B. Fauconnier, and R.-L. Girre *Antiviral Res.*, **8**, 13 (1987).
2. M. Amoros, B. Fauconnier, and R.-L. Girre, *Planta Med.*, **54**, 128 (1988).
3. C.M.O. Simões, M. Amoros, and R.-L. Girre, "Abstracts," The Brazilian-Sino Symposium on Chemistry and Pharmacology of Natural Products, Rio de Janeiro, Brazil, 1989, p. 7.
4. D.J. Rodriguez, J. Chulia, C.M.O. Simões, M. Amoros, A.M. Mariotte, and R.-L. Girre, *Planta Med.*, **56**, 59 (1990).
5. M. Kaij-A-Kamb, "Contribution à l'étude des plantes antivirales: action antiherpétique et antipoliomyélitique de la Centaurée Noire (*Centaurea nigra* L.)," Ph.D. Thesis, Faculty of Pharmacy, University of Rennes I, France, 1989.
6. W. Cutting, R. Dreisbach, and B. Neff, *Stanford Med. Bull.*, **7**, 137 (1949).
7. H. Ishitsuka, C. Ohsawa, T. Ohiwa, I. Umeda, and Y. Suhara, *Antimicrob. Agents Chemother.*, **22**, 611 (1982).
8. Y. Ninomiya, M. Aoyama, I. Umeda, Y. Suhara, and H. Ishitsuka, *Antimicrob. Agents Chemother.*, **27**, 595 (1985), and references cited therein.
9. A.L.M. Ahmad, A.B. Dowsett, and D.A.J. Tyrrell, *Antiviral Res.*, **8**, 27 (1987).
10. M. Tisdale and J.W.T. Selway, *J. Antimicrob. Chemother.*, **14** (A), 97 (1984), and the references cited therein.
11. L.M. Van Hoof, D.A. Vanden Berghe, G.M. Hatfield, and A.J. Vlietinck, *Planta Med.*, **50**, 513 (1984).
12. R. Vrijsen, L. Everaert, L.M. Van Hoof, A.J. Vlietinck, D.A. Vanden Berghe, and A. Boeyé, *Antiviral Res.*, **7**, 35 (1987).
13. J.M. Lopez Pila, H. Kopecka, and D.A. Vanden Berghe, *Antiviral Res.*, **11**, 47 (1989), and the references cited therein.
14. J. Gleye, C. Moulis, and M.N. Doazan, *Plant. Med. Phytother.*, **17**, 92 (1983).
15. M.-Th. Fauvel, J. Gleye, C. Moulis, F. Blasco, and E. Stanislas, *Phytochemistry*, **20**, 2059 (1981).
16. D.A. Vanden Berghe, A.J. Vlietinck, and L.M. Van Hoof, *Bull. Inst. Pasteur*, **84**, 101 (1986).
17. A.G. Suganda, M. Amoros, R.-L. Girre, and B. Fauconnier, *J. Nat. Prod.*, **46**, 626 (1983).
18. L.J. Reed and H. Muench, *Am. J. Hyg.*, **27**, 493 (1938).
19. D.A. Vanden Berghe, M. Ieven, F. Mertens, and A.J. Vlietinck, *J. Nat. Prod.*, **41**, 463 (1978).
20. R.W. Sidwell and J.H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).
21. Y. Tsuchiya, M. Shimizu, Y. Hiyama, K. Itoh, Y. Hashimoto, M. Nakayama, T. Horie, and N. Morita, *Chem. Pharm. Bull.*, **33**, 3881 (1985).
22. A.J. Vlietinck, D.A. Vanden Berghe, L.M. Van Hoof, R. Vrijsen, and A. Boeyé, *Prog. Clin. Biol. Res.*, **213**, 537 (1986).

Received 28 November 1989