(5.5), a precipitate occurred immediately in a solvent system composed of water alone. Precipitate formation was finally avoided by the use of a solvent mixture of 60% propylene glycol in water. To check for the effect of solvent system throughout the test pH range, the propylene glycol–water mixture was used again in the cationic and anionic pH ranges. This evaluation as to possible solvent effects that might alter LD_{50} determinations revealed that the solvent mixture, as employed in this study, had little or no apparent influence on the absorption, as reflected by the toxicity data. Therefore, the data obtained using the propylene glycol–water solution could be pooled with the aqueous data to yield results indicative of alterations in the toxicological characteristics of tetracycline.

REFERENCES

(1) T. Loomis, "Essentials of Toxicology," 1st ed., Lea & Febiger, Philadelphia, Pa., 1968, pp. 13–24.

(2) G. Levy and W. Barr, *Ration. Drug Ther.*, **6**, 1(1972).

(3) J. H. Cutts and I. G. Walker, *Cancer Res.*, **26** (Part 1), 1386(1966).

(4) B. E. Ballard, *J. Pharm. Sci.*, **57**, 356(1968).

(5) T. Loomis, "Essentials of Toxicology," 1st ed., Lea & Febiger, Philadelphia, Pa., 1968, pp. 57–69.

(6) J. L. Colaizzi and P. R. Klink, *J. Pharm. Sci.*, **58**, 1184(1969).

(7) J. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99(1949).

(8) Staff of Division of Pharmacology, "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics," 2nd ed., Association of Food and Drug Offices of the United States, Topeka, Kan., 1965, pp. 16–25.

(9) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1970, p. 7.

(10) A. Albert, *Pharmacol. Rev.*, **4**, 136(1952).

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Antileukemic and Other Constituents of *Tithonia tagitiflora* Desf.

RAGHWENDRA PAL, D. K. KULSHRESHTHA, and R. P. RASTOGI [x]

Abstract □ Phytochemical investigation of *Tithonia tagitiflora* has led to isolation of six new germacranolides, tagitinins A, B, C, D, E, and F, β-sitosterol, and its β-D-glucoside. Among these, tagitinin F possesses antileukemic activity.

Keyphrases □ *Tithonia tagitiflora*—whole plant alcoholic extract, isolation of six new germacranolides, β-sitosterol, and its β-D-glucoside, screened for antileukemic activity □ Germacranolides—six new tagitinins isolated from whole plant extract of *Tithonia tagitiflora*, screened for antileukemic activity □ β-Sitosterol and its β-D-glucoside—isolated from whole plant extract of *Tithonia tagitiflora* □ Antileukemic agents, potential—constituents isolated from whole plant extract of *Tithonia tagitiflora* screened

Tithonia tagitiflora Desf. (Compositae) is an American plant which has become naturalized in the Khandala region. Only one species of this genus, namely *T. tubaeformis*, has undergone chemical investigation, resulting in the characterization of a new flavone, tithonine (1), and a novel germacranolide, orizabin (2).

In the screening program for tumor inhibitors from plant sources (3), the alcoholic extract of *T. tagitiflora* showed significant activity against P-388 lymphocytic leukemia (PS). The results of the subsequent studies leading to the isolation of the various constituents together with their biological data are described here[1].

[1] The plant material was identified as *Tithonia tagitiflora* Desf. (Compositae) by Mr. B. N. Mehrotra, Section of Botany, Central Drug Research Institute, Lucknow, India. A voucher (preserved) specimen (No. 2499), representing material collected for this investigation, is available for inspection at the Herbarium of the Institute.

EXPERIMENTAL

The reported melting points are uncorrected. Spots on TLC were visualized with 1% ceric sulfate in 2 *N* H_2SO_4 using silica gel plates and with alkaline potassium permanganate using neutral alumina plates.

Isolation of Constituents—The alcoholic extract of the dried plant material (11.0 kg) was extracted with benzene. The residual portion was partitioned with 1-butanol–water. The benzene-soluble material was defatted with *n*-hexane, and the insoluble fraction was decolorized with charcoal in ethanol, which yielded a light-green viscous mass (165 g). This residue showed eight spots on TLC which were designated as α (R_f 0.55 in ethyl acetate saturated with water + 10% methanol), A [R_f 0.44 in chloroform–ethyl acetate (1:1)], B, C, D, E, F, and G [R_f 0.22, 0.27, 0.33, 0.40, 0.47, and 0.58, respectively, in benzene–ethyl acetate (2:1)] on neutral alumina plates. A portion of the residue (68 g) was subjected to a gross fractionation over silica gel (1 kg) as shown in Table I.

The residue from fractions 3–5, on repeated crystallization from methanol, gave substance G, mp 136° (4.0 g).

The residue from fraction 7 (7.7 g) was rechromatographed over neutral alumina (activity of 2.5, 350 g). The residue (1.5 g) from the benzene–ethyl acetate (9:1) eluate crystallized from hexane–benzene, mp 128–130° (tagitinin F, 0.75 g), and the benzene–ethyl acetate (7:3) eluate (1.44 g) crystallized in a similar manner to yield tagitinin E, mp 208–210°, 0.50 g.

Fraction 9 (Table I, 4.2 g) was rechromatographed over neutral alumina. The benzene–ethyl acetate (7:3) eluate (1.11 g), on repeated crystallization from benzene–hexane, gave tagitinin D, mp 138–140° (0.45 g). The residue from the benzene–ethyl acetate (3:2) eluate (0.80 g) was rechromatographed over silica gel impregnated with silver nitrate (12%), and the benzene–ethyl acetate (3:2) eluate yielded pure tagitinin C (0.33 g) as colorless powder.

Rechromatography of fraction 10 (Table I, 5.0 g) over neutral alumina yielded a benzene–ethyl acetate (1:1) eluate (1.15 g), which crystallized from acetone–isopropyl ether, mp 125–126° (tagitinin

Table I—Chromatography of the Defatted Benzene-Soluble Fraction (68 g)

Fraction Number	Eluant	Weight, g	TLC[a]
1—2	Benzene	1.4	St
3—5	Benzene—ethyl acetate (4:1)	10.0	St, G
6	Benzene—ethyl acetate (3:1)	8.7	G, F (E)
7	Benzene—ethyl acetate (3:1)	7.7	F, E (D), (G)
8	Benzene—ethyl acetate (2:1)	4.9	D, (E), (C)
9	Benzene—ethyl acetate (2:1)	4.2	D, C, (B), St
10	Benzene—ethyl acetate (1:1)	5.0	B, (C), (A)
11	Benzene—ethyl acetate (1:1)	2.6	A, (B)
12	Benzene—ethyl acetate (1:2)	4.8	A, St
13	Benzene—ethyl acetate (1:2)	2.8	A, (α), St
14—15	Benzene—ethyl acetate (1:3)	6.8	A, α, St
16—17	Ethyl acetate	3.2	α, St
18—19	Ethyl acetate—ethanol (9:1)	3.5	St

[a] St = streaking; spots in parentheses indicate minor constituents.

B, 0.55 g). Similarly, the residue from fractions 14–15 (Table I, 6.8 g) was rechromatographed over silica gel. The benzene–ethyl acetate (1:3) eluate (5.34 g) crystallized from chloroform–hexane, mp 168–170° (tagitinin A, 4.7 g), and ethyl acetate eluate (0.66 g) gave substance α, mp 277–278° (0.28 g).

Tagitinins A, B, and D–F were obtained as colorless needles, and C was an amorphous powder. When heated with hydroxylamine hydrochloride, they gave a magenta color. The yields of the chemical constituents and their physical data are given in Table II.

Biological Activity—Anticancer screening against P-388 lymphocytic leukemia (PS) in mice was carried out according to the National Institutes of Health protocol (4). The activity data of the total alcoholic extract (A), the defatted benzene-soluble fraction (B), and tagitinin F are given in Table III. Other fractions of the extract and the remaining tagitinins (A–E) did not show any activity. The butanol-soluble fraction showed marginal activity only at a dose of 35 mg/kg (T/C 130).

DISCUSSION

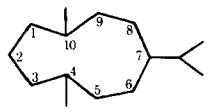
The alcoholic extract of the plant (A) was fractionated into benzene- and butanol-soluble fractions. The benzene-soluble fraction was defatted with petroleum ether, whereby the total activity was found to be localized in the insoluble residue (B, Table III). Fraction B was subjected to repeated chromatography over silica gel and neutral alumina, resulting in the isolation of eight substances designated as α, A, B, C, D, E, F, and G in order of increasing R_f values. Substances A–F, being new, are named as tagitinins.

Table III—Activity of Plant Extract and Its Fractions in PS System

Materials Tested	Dose, mg/kg	Survivors[a], Number Taken on Day 5	Median Survival Time, days, Treated/Control	T/C[b], %	Status
Alcoholic extract A	800	6/6	13.3/10.0	133	Active
	400	6/6	14/10.0	140	Active
NSC No. B67 1964	200	6/6	15/10.0	150	Active
	100	6/6	14.4/10.0	144	Active
	50	6/6	11.8/10.0	118	Inactive
	25	6/6	10.6/10.0	106	Inactive
Fraction B	40	3/6	—	—	Toxic
	20	6/6	12.7/10.0	127	Active
	10	5/6	12.1/10.0	121	Inactive
	5	6/6	13.3/10.0	133	Active
	2	6/6	10.0/10.0	109	Inactive
Tagitinin F	50	4/4	16.1/10.0	161	Active
	25	4/4	15.5/10.0	155	Active
	12.5	4/4	16.1/10.0	161	Active

[a] Number is a measure of toxicity; 4/6 or 66.6% survival is considered to be nontoxic. [b] Treated/control (T/C) is also survival time of the test mice with respect to the survival of control mice. It is expressed as percent obtained by: (median survival time of treated mice/median survival time of control mice) × 100. A T/C value greater than 125 is considered as active.

All of these tagitinins were bitter in taste and gave a positive hydroxamic acid test for an ester or lactone group. All of them exhibited characteristic IR bands in the regions of 1760 cm^{-1} for α,β-unsaturated-γ-lactone and 1730 cm^{-1} for an ester grouping. In addition, they showed strong UV absorption characteristics of an α,β-unsaturated-γ-lactone chromophore (Table II).



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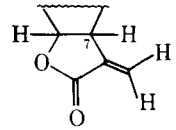

II

On the basis of physicochemical studies, they were found to be sesquiterpenoids, having the germacrane skeleton (I), an α,β-unsaturated-γ-lactone with an *exo*-methylene (II), and isobutyric ester groupings at C-6–C-8 and C-8–C-6. The differences were only in the oxygenation pattern and unsaturation at C-1, C-2, C-3, C-4, C-5, and C-10. Only tagitinin F showed antitumor activity in the P-388 system.

Table II—Substances Isolated from *T. tagitiflora*

Substance	Molecular Formula[a]	Melting Point ($[\alpha]_D$)	IR, cm^{-1}	UV, nm	Yield[b], %
Tagitinin A[c]	$C_{19}H_{28}O_7$	168—170° (−154°)	1757, 1732 1665, 890	215 (11,420)	0.1068
Tagitinin B[c]	$C_{19}H_{26}O_7$	125° (−142°)	1755, 1727 1642, 1157 890	214 (12,993)	0.0124
Tagitinin C[c]	$C_{19}H_{24}O_6$	Amorphous (−204°)	1757, 1727 1665, 886	212, 248 (16,280, 10,842)	0.0091
Tagitinin D[c]	$C_{19}H_{28}O_6$	138—140° (−137°)	1770, 1720 1665, 890	218 (18,900)	0.0101
Tagitinin E[c]	$C_{19}H_{26}O_6$	207—210° (−101°)	1760, 1730 1655, 890	213 (9,460)	0.1230
Tagitinin F[c]	$C_{19}H_{24}O_6$	128—130° (−144°)	1760, 1730 1655, 877	215 (12,240)	0.0173
α (β-sitosterol-β-D-glucoside)	$C_{35}H_{60}O_6$	277—278° (−45°)	—	—	0.0063
G (β-sitosterol)	$C_{29}H_{50}O$	136° (−39°)	—	—	0.0045

[a] Molecular formulas were deduced by carbon–hydrogen analyses and mass spectra (M^+). [b] Based on the weight of dry plant. [c] Indicates new substance.

REFERENCES

(1) J. Correa and M. L. Cervera, *Bull. Soc. Chim. Fr.*, **1971**, 475.

(2) A. Romo de Vivar, J. Romo, A. A. Ortega, E. Diaz, and M. A. Carino, *Rev. Latinoam. Quim.*, **2**, 38(1971).

(3) D. S. Bhakuni, M. L. Dhar, M. M. Dhar, B. N. Dhawan, B. Gupta, and R. C. Srimal, *Indian J. Exp. Biol.*, **9**, 91(1971).

(4) *Cancer Chemother. Rep.*, **3**, 1(1972).

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Conformationally Defined Adrenergic Agents I: Potentiation of Levarterenol in Rat Vas Deferens by *endo*- and *exo*-2-Aminobenzobicyclo[2.2.2]octenes, Conformationally Defined Analogs of Amphetamine

GARY L. GRUNEWALD [*x], JAMES A. RUTH [*], TIMOTHY R. KROBOTH [*], BHARAT V. KAMDAR [*], POPAT N. PATIL [‡], and KADIM N. SALMAN [‡]

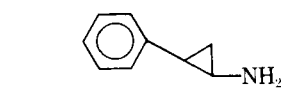
Abstract □ Four conformationally defined analogs of amphetamine were synthesized and studied for their ability to potentiate the action of levarterenol on the isolated vas deferens from reserpine-treated rats. The compounds also were studied for indirect adrenergic agonist activity in the same test system. A definite stereochemical correlation was demonstrated in each case, the *exo*-isomers being considerably more active than their *endo*-counterparts both in potentiation and in indirect activity. The more active isomers of each pair correspond to an *anti*-periplanar conformation of amphetamine. The compounds are probably acting by inhibition of the neuronal amine uptake mechanism, since none of the compounds was a direct-acting agonist itself. These results are discussed in relationship to other previously reported, conformationally defined, amphetamine analogs.

Keyphrases □ Adrenergic agents—conformationally defined analogs of amphetamine, potentiation of levarterenol in rat vas deferens □ Levarterenol—potentiation by conformationally defined analogs of amphetamine, rat vas deferens □ Amphetamines—conformationally defined analogs synthesized, potentiation of levarterenol in rat vas deferens □ Structure–activity relationships—conformationally defined analogs of amphetamine, potentiation of levarterenol in rat vas deferens

One recent technique for probing the nature of agonist–receptor interactions is the study of conformationally defined analogs of the agonists in question to quantitate the stereochemical requirements for biological activity. While such an approach has found considerable application in the areas of cholinergic agonists (1–5), on phenethanolamine systems (6–11), and dopaminergic agonists (12), relatively few examples of conformationally defined analogs of amphetamine have been reported and definite structure–activity relationships are noticeably scant.

Tranylcypromine (*trans*-2-phenylcyclopropylamine) (I) was considerably more potent than the *cis*-isomer (II) in its ability to inhibit catecholamine uptake into synaptosomes from the rat hypothalamus and corpus striatum (13). Similarly, 2-aminoindan (III) was more efficacious than 1-aminoindan (IV) in the same experiments. These results suggested that the fully extended, or *anti*-periplanar, conformation of amphetamine was the important spatial disposition necessary for inhibition of the neuronal uptake mechanism.

The synthesis of a series of 2-amino-3-phenyl-*trans*-decalins (V–VIII) was reported (14). Surprisingly, all of these compounds decreased the motor activity in mice at similar dosage levels so that no correlation of activity with stereochemistry could be discerned. The compounds were also surveyed (15, 16) as inhibitors of 5-hydroxytryptamine and histamine uptake in rabbit



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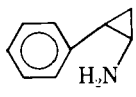

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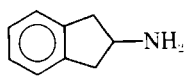

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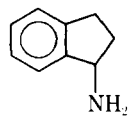

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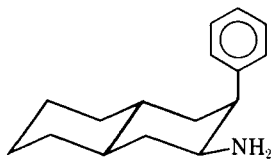

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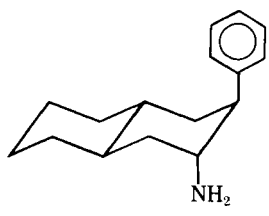

VI