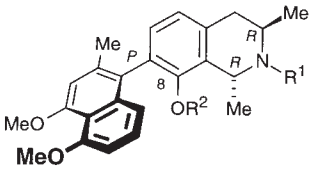

	R^1	R^2	LD_{100}
5	H	Me	40 ppm
6	H	Et	20 ppm
7	H	*i*-Pr	10 ppm
8	H	Bzl	10 ppm
9	CHO	Me	10 ppm
10	CHO	*i*-Pr	20 ppm
11	CHO	Ac	> 40 ppm
12	CHO	Bz	> 40 ppm
13	CHO	H	> 40 ppm
14	Ac	H	> 40 ppm
15	Me	H	> 40 ppm

Fig. 2 Synthetic derivatives of dioncophylline A(**1**).

nificant activities. Work aiming at the isolation or, respectively, preparation and testing of further natural or chemically modified representatives of this intriguing class of biaryl alkaloids, is underway.

Materials and Methods

Samples of *Ancistrocladus abbreviatus* Airy Shaw and *A. barteri* Scott Elliot (Ancistrocladaceae) and of *Triphyophyllum peltatum* (Hutch. & Dalz.) Airy Shaw (Dioncophyllaceae) were collected and identified by L. Aké Assi in West Ivory Coast in January 1988; voucher specimens are deposited at the Conservatoire et Jardin Botaniques de l'Université d'Abidjan, Ivory Coast. Samples of *Ancistrocladus tectorius* (Lour.) Merr. (Ancistrocladaceae) were collected and botanically determined by C. Zhao in P. R. China; voucher specimens are kept at the Hainan Provincial Hospital, Haikou, P. R. China. The air-dried, ground plant material was extracted with petroleum ether, then with CH_2Cl_2, and subsequently with CH_2Cl_2/NH_3; naphthylisoquinoline alkaloids were isolated as described previously (2) by gradient column chromatography on silica gel. Derivatives were synthesized using established, highly selective reaction sequences. The molluscicidal activity against *Biomphalaria glabrata* was performed as published earlier (7). Detailed information on the isolation and synthetic work and the test system is obtainable from the authors of correspondence.

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Diterpenes and Synthetic Derivatives from *Viguiera aspillioides* with Trypanomicidal Activity

Fernando B. da Costa[1], Sérgio Albuquerque[1,2], and Walter Vichnewski[1]

[1] Departamentos de Física e Química e Ciências da Saúde, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n, 14040-903, Ribeirão Preto-SP, Brazil
[2] Address for correspondence

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Abstract: **The dichloromethane extract of the tuberous roots of *Viguiera aspillioides* was tested *in vitro* against *T. cruzi* and then investigated in order to identify its active compounds, which were the known diterpenes (−)-*ent*-kaur-16-en-19-oic acid, (−)-trachyloban-19-oic acid, and (−)-kauran-16α-ol. Synthetic derivatives of the acidic compounds were obtained and tested; one of them, (−)-kaur-16-en-19-ol, was also active. Their IC_{50} are given.**

The American trypanosomiasis caused by *Trypanosoma cruzi* (Chagas' disease) is a serious problem for health services in urban centers of the American continent because of infection of patients through blood transfusions. Besides blood tests, addition of chemical substances to stored blood is one prophylactic way which may prevent this mechanism of transmission. Gentian violet (1) is the only substance employed for this purpose although it may cause undesirable effects in the patients (2).

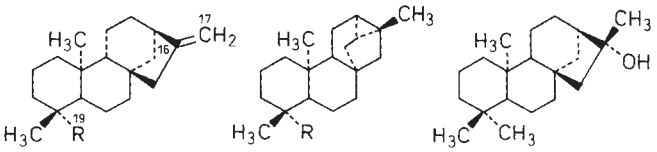
In our laboratory, several plant extracts from Asteraceae were screened *in vitro* against trypomastigotes blood forms of

T. cruzi. The CH_2Cl_2 extract of the tuberous roots of *Viguiera aspillioides* has shown good results in the bioassays and some active compounds have been isolated and identified. Some of their synthetic derivatives were also tested *in vitro* in order to evaluate their trypanomicidal activity. The IC_{50} of all compounds was determined.

Viguiera aspillioides Gardn. (Asteraceae: Heliantheae) was collected at Fazenda do Turvo, near Furnas, MG, Brazil, in November 1989 and identified by Prof. Hermógenes F. L. Filho, from the Universidade Estadual de Campinas, SP. A voucher specimen is deposited in the herbarium of the Instituto de Biologia at the same university, under the number UEC-22.911. The dried and powdered tuberous roots of *V. aspillioides* (64.0 g) were exhaustively extracted with CH_2Cl_2 at room temperature. The solvent was evaporated and 6.4 g of crude extract were obtained.

The CH_2Cl_2 extract was chromatographed over 150 g of silica gel D (Riedel de Häen, Germany) by vacuum liquid chromatography (3) and eluted with mixtures of hexane and increasing amounts of EtOAc (5 % steps up to 1 : 1 and then EtOAc) yielding 16 fractions of 250 ml. After analyzing the fractions by TLC (silica gel, hexane/EtOAc, 7.5 : 2.5) they were recombined according to TLC similarity and the number reduced to 10. The fractions that showed trypanomicidal activity on the *in vitro* bioassays were fractions 2 (2.4 g, hexane-EtOAc, 9 : 1), 3 (0.9 g, hexane-EtOAc, 9 : 1), and 4 (1.26 g, hexane-EtOAc, 4 : 1). An aliquot of fraction 2 (ca. 600 mg) was submitted to chromatography on Sephadex LH-20 (15 g) and eluted with hexane-CH_2Cl_2-MeOH, 7 : 4 : 1. Similar fractions (tested by TLC, as above) were combined and, after recrystallization from MeOH, about 250 mg of (−)-trachyloban-19-oic acid (**2a**) were obtained as white crystals, m.p. 93–96 °C, $[\alpha]_D^{25}$: −47.58° (*c* 0.29, $CHCl_3$) (4). After successive recrystallizations from MeOH, fraction 3 afforded about 700 mg of (−)-*ent*-kaur-16-en-19-oic acid (**1a**) as colorless prisms, m.p. 179–181 °C, $[\alpha]_D^{25}$: −109.60° (*c* 1.5, $CHCl_3$) (5–7). An aliquot of fraction 4 (ca. 600 mg) was treated like fraction 2 (above) yielding 20 mg of (−)-*ent*-kauran-16α-ol (**3**), as white leaflets, m.p. 182–184 °C, $[\alpha]_D^{25}$: −24.61° (*c* 0.13, $CHCl_3$) (6, 8).

About 150 mg of **1a** and **2a** were treated with CH_2N_2 in Et_2O yielding their respective methyl ester derivatives, (−)-methyl kaur-16-en-19-oate (**1b**), white leaflets, m.p. 72–75 °C, $[\alpha]_D^{25}$: −91.9° (*c* 7.93, $CHCl_3$) (5, 7, 9) and (−)-methyl trachyloban-19-oate (**2b**), white solid, m.p. 93–95 °C, $[\alpha]_D^{25}$: −68.48° (*c* 1.55, $CHCl_3$) (4, 9, 10). Then 100 mg of the methyl esters **1b** and **2b** were treated with excess $LiAlH_4$ and the respective C-19 alcohol derivatives, (−)-kaur-16-en-19-ol (candol B, **1c**), m.p. 134–138 °C, $[\alpha]_D^{25}$: −51.64° (*c* 1.46, $CHCl_3$) (9, 11) and (−)-trachyloban-19-ol (**2c**), m.p. 126–129 °C $[\alpha]_D^{25}$: −37.63°, (*c* 0.93, $CHCl_3$) (9, 10) were obtained as white leaflets. About 40 mg each of the C-19 alcohol derivatives **1c** and **2c** were treated with excess of acetic anhydride in pyridine to give the C-19 acetoxyl derivatives (−)-19-acetoxy-kaur-16-ene (**1d**), m.p. 104–108 °C, $[\alpha]_D^{25}$: −61.48° (*c* 0.54, $CHCl_3$) (9, 11) and (−)-19-acetoxy-trachylobane (**2d**), m.p. 98–104 °C, $[\alpha]_D^{25}$: −31.59° (*c* 0.88, $CHCl_3$) (9, 10), both as white needles. All derivatives were recrystallized from MeOH and their spectral data as well as synthetic preparation techniques are in full agreement with those reported in the references cited above. Copies of the original spectra are obtainable from the author of correspondence.



	R		R	3
1a	CO_2H	**2a**	CO_2H	
1b	CO_2CH_3	**2b**	CO_2CH_3	
1c	CH_2OH	**2c**	CH_2OH	
1d	CH_2OAc	**2d**	CH_2OAc	

The bioassays of all compounds were carried out using blood collected by cardiac puncture of Swiss albino mice in the parasitemy peak (7th day) after infection with the Y strain of *T. cruzi*. The blood was diluted with normal murine blood to give a concentration of ca. 2×10^6 trypomastigote forms/ml. Stock solutions of the compounds to be tested were prepared by dissolution in DMSO (dimethyl sulfoxide) to a final concentration of 25 mg/ml. The bioassays were performed in triplicate on microtitre plates (96 wells) which contained 400 μl of mixture/well. To each sample compound, aliquots of the stock solutions were added to the diluted blood in such quantities as to give final concentrations of 100, 200, 500 and 1000 μg of compound per ml of mixture in the wells. The plates were incubated at 4 °C during 24 h and the number of parasites determined according to Brener (12). The crude extract was assayed in the same way, except that the solution in DMSO was 4 mg/ml and the final concentration 1600 μg/ml. Controls were blood of infected mice without any addition, infected blood containing DMSO in equivalent amounts as the samples, and infected blood containing gentian violet (positive control) at a concentration of 250 μg/ml.

Table **1** shows lysis percentages of *T. cruzi* trypomastigote forms induced by addition, to the infected blood, of the crude extract of *V. aspillioides*, the isolated compounds, or their synthetic derivatives. The lowest IC_{50} value was obtained for compound **1c** (200 μg/ml; 0.69 mM). Compounds **1a**, **2a**, and **3** showed IC_{50} values of 500 μg/ml (1.66, 1.66, and 1.72 mM, respectively). For the other derivatives the IC_{50} values were higher. By comparison, the more active compounds were approximately 10 to 20 times less active than gentian violet (IC_{50} = 76 μM).

This is a first report of *in vitro* trypanomicidal activity of (−)-trachyloban-19-oic acid (**2a**), (−)-kauran-16α-ol (**3**), and (−)-kaur-16-en-19-ol (**1c**). It has been recently reported that (+)-*ent*-kaur-16-en-19-oic acid from *Mikania obtusata* D.C. exhibits *in vitro* trypanomicidal activity (13). In the present study, we relate the same activity for (−)-*ent*-kaur-16-en-19-oic acid (**1a**) whose physical and spectral data are identical with those reported in the literature (5, 6, 7). In addition, all natural diterpenes from *V. aspillioides* and their synthetic derivatives have shown the same sign for specific rotation. So, if it is assumed that the kaurenoic acid from *V. aspillioides* is an enantiomer of that from *M. obtusata* (13), it is the first report of such activity for the (−)-enantiomer.

Notwithstanding the rather high values of IC_{50} for our active compounds it is possible to visualize some aspects which could

Table 1 Lysis percentages of *T. cruzi* trypomastigotes forms by diterpenes from *V. aspillioides* and their synthetic derivatives performed in *in vitro* bioassays.

Compound	Dose (μg/ml) × Trypanomicidal Activity (lysis %)[a,b,c] 100	200	500	1000	1600
Extract	–	–	–	–	75 ± 7
1a	32 ± 6	41 ± 3	53 ± 8	100	–
1b	15 ± 5	23 ± 6	39 ± 10	60 ± 7	–
1c	24 ± 9	47 ± 7	68 ± 5	100	–
1d	13 ± 2	25 ± 8	32 ± 4	48 ± 6	–
2a	25 ± 7	39 ± 9	49 ± 3	100	–
2b	10 ± 5	12 ± 6	23 ± 9	33 ± 10	–
2c	8 ± 9	17 ± 5	29 ± 7	49 ± 4	–
2d	2 ± 12	6 ± 9	12 ± 5	17 ± 8	–
3	27 ± 3	38 ± 6	51 ± 8	100	–

[a] Percent reduction of the parasite number in mice infected blood.
[b] Gentian violet, the positive control, has IC_{50} = 76 μM and was used at a 250 μg/ml concentration.
[c] The controls, mice infected blood without any added compound and mice infected blood containing the same DMSO concentration used in the stock solutions, have not showed any reduction of the parasite numbers.

be considered in regard to SAR (structure-activity relationship). Compounds **1a** and **1c** bearing polar groups (-COOH and -CH_2OH) at C-19 had marked trypanomicidal activity in contrast to derivatives **1b** and **1d**. It seems, then, that the activity of these kaurene derivatives depends on the presence of polar groups at C-19. The activity of compound **2a** also seems to confirm this assertion. No conclusion can be drawn with respect to compound **3**, whose structure has neither a C-16,17 double bond nor C-19 polar groups. All these observations have led us to propose that other synthetic derivatives of **1a** and **2a** should be obtained and tested *in vitro* against *T. cruzi* in order to obtain compounds of higher activity and thus better understand their SAR.

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A Novel Stilbene Glucoside, Oxyresveratrol 3′-*O*-*β*-Glucopyranoside, from the Root Bark of *Morus alba*

Feng Qiu[1], Kenichi Komatsu[1], Kanako Kawasaki[1], Kenichi Saito[1], Xinsheng Yao[2], and Yoshihiro Kano[1,3]

[1] Department of Kampo Medicinal Science, Hokkaido College of Pharmacy, 7-1 Katuraoka-cho, Otaru 047-02, Japan
[2] Department of Phytochemistry, Shenyang Pharmaceutical University, 103 Wenhua road, Shenyang, China
[3] Address for correspondence

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Abstract: **A novel stilbene glucoside was isolated from the root bark of *Morus alba* L. (Moraceae), along with mulberroside A, *cis*-mulberroside A, oxyresveratrol. The structure of the novel stilbene glucoside was determined as oxyresveratrol 3′-*O*-*β*-glucopyranoside.**

Morus alba L. (Moraceae) (sangbaipi in Chinese) is mainly distributed in the Anhui, Hunan, and Zhejiang provinces in China. Its root bark is usually used as an expectorant, a diuretic, and a laxative (1) in traditional Chinese medicine. During our studies on the bioactive constituents of *M. alba*, we isolated four stilbene constituents of which one was unknown. In this report, we describe the isolation and structural determination of the novel stilbene, oxyresveratrol 3′-*O*-*β*-glucopyranoside (**3**), along with other three known constituents, mulberroside A (**1**), *cis*-mulberroside A (**2**), and oxyresveratrol (**4**).

Compound **3** was obtained as a white powder. The positive SI-MS spectrum of **3** showed the pseudomolecular ion peak at *m/z* = 407 $[M + 1]^+$, indicating a molecular formula of $C_{20}H_{22}O_9$,

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