

Cytotoxic Activity of Polyindoline Alkaloids of *Psychotria forsteriana* (Rubiaceae) (1)

A. Roth¹, B. Kuballa¹, C. Bounthanh¹, P. Cabalion², T. Sévenet³, J. P. Beck⁴, R. Anton^{1,5}

Received: April 30, 1986

Abstract: We have shown that the planar formula of the polyindoline alkaloids (C, B, E and D) isolated from the leaves of *Psychotria forsteriana* agree respectively with Quadrigemine A, Quadrigemine B, Psychotridine, and Isopsychotridine C. Their cytotoxic activity on cultured rat hepatoma cells (HTC line) is reported in this paper. These alkaloids showed a higher toxicity on HTC cells than vincristine, a bisindole alkaloid currently used in antitumor chemotherapy.

Introduction

Psychotria forsteriana A. Gray is a tropical plant growing in Vanuatu (New Hebrides). In a previous paper (2), we reported the isolation of four major alkaloids from the leaves of this shrub. New spectral data concerning their planar structures allow us to correlate them respectively with the stereoisomeric groups of Quadrigemine A (alkaloid C), Quadrigemine B (alkaloid B), Psychotridine (alkaloid E), and Isopsychotridine C (alkaloid D) (Figure 1). These alkaloids are polyindoline compounds resulting from the condensation of several N_b -methyltryptamine units. At the present time, polymers including 3, 4, or 5 units seem to be specific for two genera of Rubiaceae (*Psychotria*, *Hodgkinsonia*) (3, 4). Obviously, this phenomenon of polycondensation is responsible for numerous possibilities of stereoisomerism on account of the great number of asymmetric carbons in these structures. Thus, (C) and (B) are two isomers composed of four N_b -methyltryptamine units whereas (E) and (D) are isomers composed of five similar units (Fig. 1).

In 1982, Libot (3) showed that such isomeric polyindoline alkaloids isolated from *P. oleoides*, were slightly cytotoxic on leukemic mice cells (L. 1210). It seemed to be of interest to test

the alkaloids extracted from *P. forsteriana* on another tumor system in order to investigate their cytotoxic properties.

In the present work, we studied the cytotoxic effects of these compounds on cultured rat hepatoma cells (HTC line) in relation to their respective concentrations. The well known bisindole alkaloid vincristine (= leurocristine), commonly used in anticancer chemotherapy, was chosen as a standard antitumor agent (5).

Materials and Methods

Chemistry

Extraction, isolation, and purification of alkaloids C, B, E, and D were described in a previous paper (2). The number of N_b -methyltryptamine units in each compound, as well as the C-C bond types (C_β - C_β or C_β -C phenyl types) and their respective positions in the molecular planar structures were determined on the basis of their spectral data in comparison with those found in the literature (2, 3, 6, 7).

Alkaloid B: $C_{44}H_{50}N_8$, $[\alpha]_D^{20}$: + 215° (EtOH); $[\alpha]_D^{20}$: - 10° (CHCl₃); UV (EtOH) λ_{max} nm (log ϵ) = 243 (4.24), 302 (3.96); IR (film/CHCl₃) ν cm⁻¹ = 3420, 3380, 3250 (NH), 1610 (indoline); MS *m/e* (abundance %) = 720 (3), 719 (4), 693 (12), 692 (45), (MH⁺) 691 (100), (M⁺) 690 (82), 689 (24), 659 (2), 658 (4), 519 (4), 518 (15), 517 (45), 516 (36), 325 (5), 221 (2), 220 (6), 219 (2), 205 (4), 173 (19), 172 (15); ¹³C-NMR (CDCl₃, TMS, shifts are given in δ ppm): C = 150.6; 149.1; 133.2; 132.9; 132.5; 63.9; 63.3; 60.9; 60.1 - CH = 128.0; 127.8; 125.9; 125.1; 124.3; 123.9; 123.8; 122.4; 119.1; 118.3; 117.2; 116.8; 116.6; 108.9; 87.1; 85.9; 83.3; 82.3 - CH₂ = 52.3; 52.2; 38.5; 36.6 - CH₃ = 35.8; 35.7; 35.6; 35.2.

Alkaloid C: $C_{44}H_{50}N_8$, $[\alpha]_D^{20}$: + 32.5° (c 0.2 EtOH); $[\alpha]_D^{20}$: + 115.7° (c 0.2, CHCl₃); UV (EtOH) λ_{max} nm (log ϵ) = 244 (4.25), 302 (4.02); IR (film CHCl₃) ν cm⁻¹ = 3420, 3260 (NH), 1610 (indoline); MS *m/e* (abundance %) = 720 (2.5), 719 (4.2), 693 (5), 692 (20), (MH⁺) 691 (50) (M⁺) 690 (35), 689 (25), 658 (2), 519 (1), 373 (3), 346 (17), 345 (82) 344 (100), 343 (5), 315 (2), 314 (7), 313 (2), 302 (4), 301 (5), 300 (2), 163 (5), 156 (5), 150 (4), 141 (4), 135 (7); ¹³C-NMR (CDCl₃, TMS, shifts are given in δ ppm): C = 150.9; 150.7; 132.6; 132.4; 132.3; 63.2; 62.9; 60.9; 60.8 - CH = 127.9; 126.4; 126.0; 125.4; 124.9; 122.5; 118.8; 118.7; 116.3; 116.2; 115.7; 109.0; 108.9; 86.9; 86.1; 82.5 - CH₂ = 52.6; 52.5; 52.2; 38.8; 38.7; 38.5; 36.6; 36.3 - CH₃ = 35.7; 35.5; 35.0.

Alkaloid E: $C_{55}H_{62}N_{10}$, $[\alpha]_D^{20}$: + 153° (c 0.35, EtOH); $[\alpha]_D^{20}$: + 49° (c 0.2, CHCl₃); UV (EtOH) λ_{max} nm (log ϵ) = 243 (4.25), 303 (4.01); IR (film CHCl₃) ν cm⁻¹ = 3420, 3380, 3260 (NH), 1710 (C = O region, impurities), 1600 (indoline); MS *m/e* (abundance %) = 892 (3), 891 (9),

¹ Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université Louis Pasteur, B. P. 10, F-67048 Strasbourg Cedex, France.

² O.R.S.T.O.M.; Noumea, New-Hebrides.

³ C.N.R.S., Institut de Chimie des Substances Naturelles, F-91190 Gif-sur-Yvette, France.

⁴ Laboratoire de Physiologie Générale, Institut de Physiologie, Université Louis Pasteur, 21 Rue Descartes, F-67000 Strasbourg, France.

⁵ Address for correspondence.

ORSTOM Fends Documentaire

N° : 30.621 2x1

17 SEP. 1990

Cote : B 2 III

865 (17), 864 (58) (MH⁺) 863 (100) (M⁺) 862 (58), 861 (25), 831 (2), 830 (3), 518 (11), 517 (55), 516 (60), 515 (3), 480 (2), 473 (3), 444 (3), 432 (3), 431 (3), 383 (3), 346 (17), 345 (72), 344 (86), 343 (5), 315 (3), 314 (10); ¹³C-NMR (CDCl₃, TMS, shifts are given in δ ppm): C = 150.7; 149.0; 133.2; 132.5; 132.3; 63.0; 60.9; 60.7; 60.1 - CH = 128.1; 127.9; 125.1; 124.0; 122.3; 118.9; 117.4; 115.9; 108.9; 86.1; 82.3 - CH₂ = 52.6; 52.4; 52.2; 52.1; 39.1; 38.5 - CH₃ = 35.7; 35.6; 35.1.

Spectral data of alkaloid D were previously described (2) and on account of the small amount of isolated alkaloids, further stereochemical identification could not be carried out.

Cytotoxic tests in vitro

Cytotoxicity tests were performed on cultured rat hepatoma cells (HTC line) derived from clone 7288 of a Morris rat hepatoma (8). The cells were cultured in suspension, in Swim's S-77 medium, complemented with 10% new born calf serum (GIBCO). The cells were incubated in 75 ml culture flasks at 37°C, under magnetic stirring. At the start, the cultures were diluted with fresh medium and adjusted to 10⁵ cells per ml. Under these conditions, the cell growth was exponential during 3 to 4 days in the same medium, with an average generation time of 28 hours, before reaching the stationary phase at a density of about 8 × 10⁵ cells per ml. The ethanolic alkaloidal solutions (50 μl per culture flask) were added at various concentrations to the cell cultures. Control experiments showed that this amount of ethanol has no effect on cell proliferation. Cell growth was measured every 24 hours, by counting the number of the cells with a Neubauer microcytometer. Samples of culture were previously incubated during 15 minutes in the presence of Trypan Blue, in order to estimate the number of viable and dead cells.

The viability of the cells was expressed as a function of time, by means of growth or survival curves. The comparison of the different

viability curves with that of a standard was used to determine the biological activity of the tested compounds, at a given concentration (inactive, cytostatic, or cytotoxic).

Results and Discussion

Fig. 2 shows the growth or survival curves of HTC cells incubated in the presence of increasing concentrations of (C), (B), (E), and (D) during three days. The experiment was carried out on the same batch of HTC cells and the viability is expressed as per cent of initial cells at the initiation of the experiments.

It can be seen that the alkaloids isolated from the leaves of *Psychotria forsteriana* are potent cytotoxic compounds. At a concentration of 5 μM, a cellular mortality of 100% was obtained within 24 hours of incubation, except with (B), the less active alkaloid which killed only 50% of the cells within the same time. In the same experimental conditions vincristine, a bisindole alkaloid well known for its antitumoral activity, at a closely related dose (8 μM/ml) (Fig. 2a) inhibited cellular growth after 24 hours and remained only cytostatic even at a 10 times higher dose (80 μM), up to the end of the experiment, without the appearance of dead cells.

A survey of the viability curves displays that the four alkaloids are unequally toxic to HTC cells. Thus, 100% cellular mortality was obtained after 24 hours at 2.5 μM for (E), 5 μM for (D) and (C), and 10 μM for (B), respectively. Nevertheless, at a dose of 2.5 μM, some discrepancy appeared between (D)

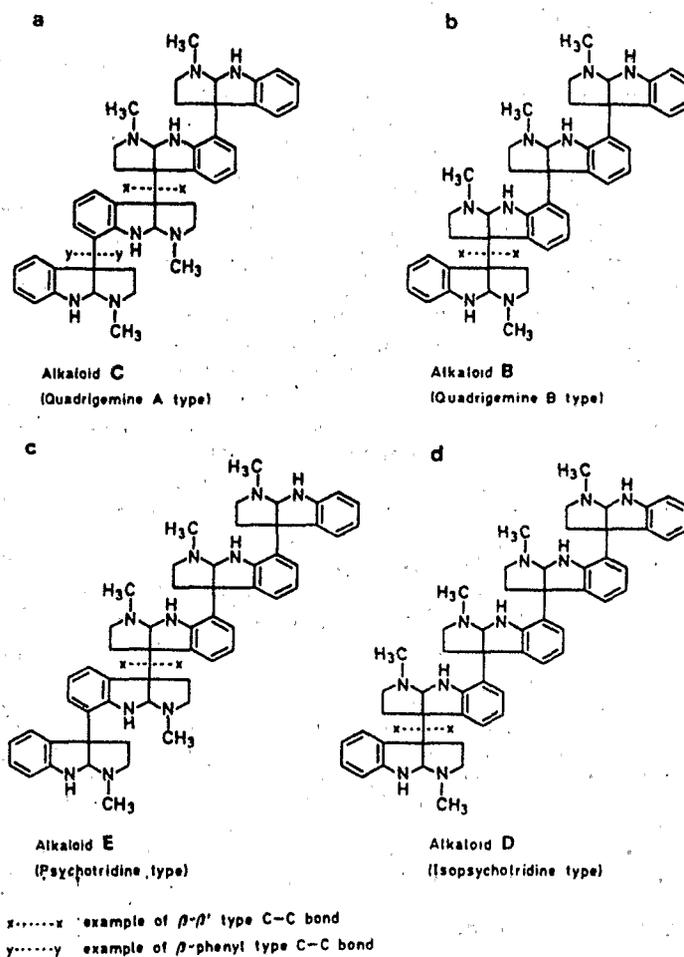


Fig. 1. Polyindoline alkaloids isolated from the leaves of *Psychotria forsteriana*

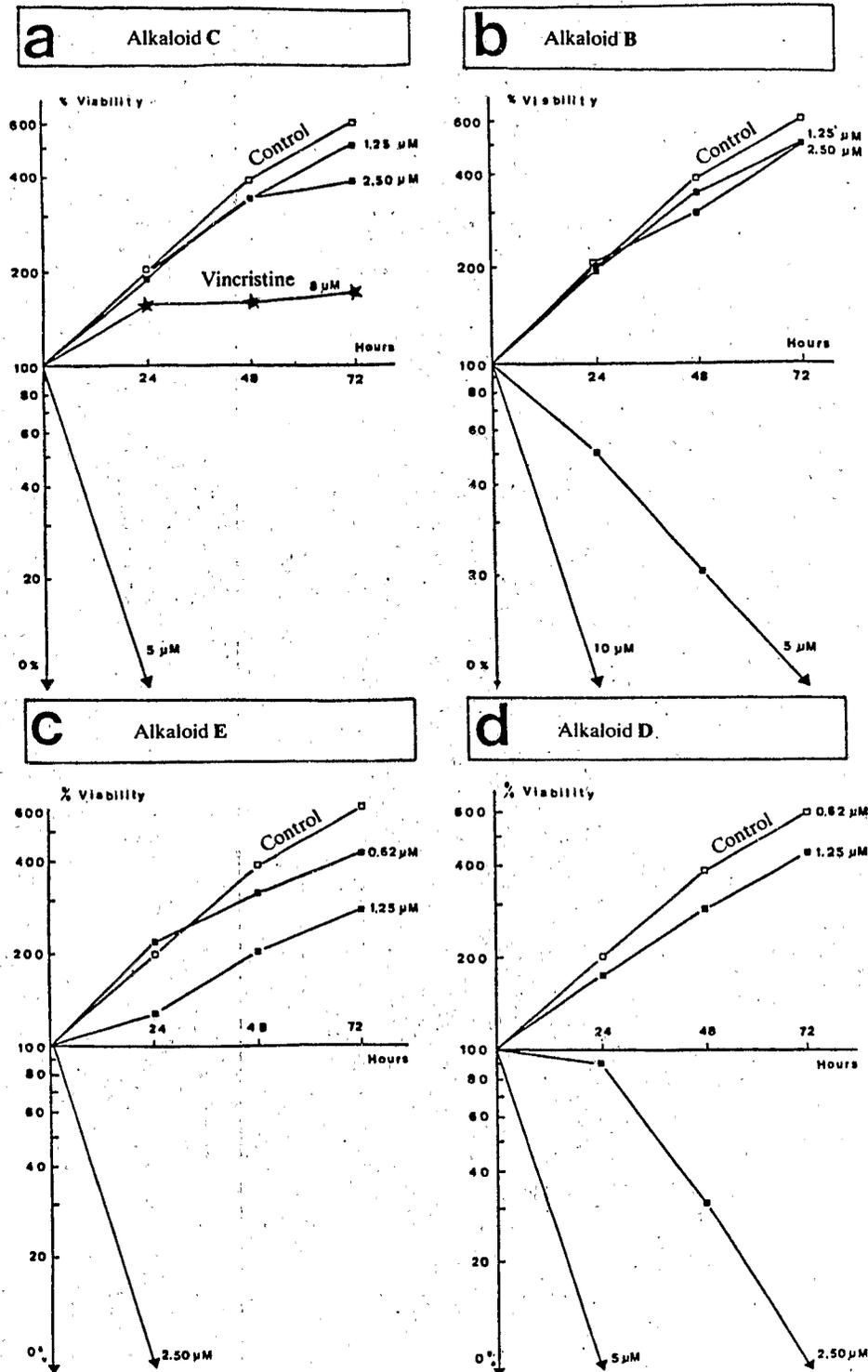


Fig. 2. Action of polyindoline alkaloids on HTC cells

The products were added to the cell suspensions at time 0. The viability of the cells is expressed as the ratio: $\frac{\text{total cells} - \text{dead cells}}{\text{initial cells}} \times 100$.

a) Effect of alkaloid C at increasing concentrations on cell growth or viability

—★—★—: effect of vincristine at a concentration of 8 μM.

b), c), d): Effect of alkaloids (B), (E), and (D), respectively, at increasing concentrations on cell growth or viability

The curves represent mean values from a typical experiment on a same batch of cells. Experiments were run 3 times in duplicate.

and (C): the first one caused a cellular mortality increasing with the incubation time (10% in 24 hours; 70% in 48 hours; 98% in 72 hours) whereas the second one remained quite inactive during the same period. Roughly, the relative cytotoxicities of these polyindoline compounds could be schematized as follows: (E) > (D) > (C) > (B).

A more detailed examination of Fig. 2 reveals that the four alkaloids could be joined in pairs, each pair having a different

behaviour towards HTC cells. Indeed, it appears that the viability curves of (D) and (B), from one side, and (E) and (C), from the other side, show some analogous aspects. For (D) and (B), a dose-effect relationship can be observed when their respective concentrations were reduced to half. Incubation in the presence of (B) at 10 μM caused a 100% cellular mortality within 24 hours; a concentration of 5 μM killed 50% of the cells in 24 hours. The viability curves of HTC cells treated by these

two doses of (**B**) are quite superimposable on those obtained with concentrations of 5 μ M and 2.5 μ M/ml of (**D**).

However, (**E**) and (**C**) did not induce the same dose-effect relationship. At concentrations of 2.5 μ M of (**E**) and 5 μ M of (**C**), all of the cells were killed within 24 hours, whereas at half these concentrations, neither (**E**) nor (**C**) showed any cytotoxic activity during the three days of the experiment. Finally, it seems that the biological activity of these compounds is not directly proportional to the doses, but corresponds virtually to a threshold effect; even the usual stage of inhibition of cell proliferation is not obvious.

Also, other analogies can be noted between the two groups of alkaloids. In each group, one of the compounds possessed a stronger cytotoxicity than the other one: thus, (**E**) and (**D**) seem to be twice as cytotoxic as (**C**) and (**B**), respectively. But, at lower concentrations which varied from 2.5 μ M to 0.62 μ M, according to the alkaloid neither cytotoxic nor cytostatic effects to HTC cells were observed with any of the four studied alkaloids. In the case of (**E**), a slightly inhibitory effect on cell growth was obtained at the dose of 1.25 μ M.

Since the stereochemical configurations of these alkaloids had not been determined, we do not have all the data necessary to discuss unambiguously their structure-activity relations. Nevertheless, the preceding growth or survival curves analysis suggests that the analogies and discrepancies which appeared between the four alkaloids on HTC cells could be partly due to their respective structural characteristics.

Thus, the most active alkaloids are (**E**) and (**D**) which contain five condensed units, whereas (**C**) and (**B**) with only four units show a comparatively lower cytotoxic effect (Fig. 2). One can also suggest that the observed cytotoxic analogies between

the pairs **E/C** and **D/B** could be due to an identical position of the $C_{\beta}-C_{\beta'}$ bond in each pair of compounds (Fig. 1).

New alkaloids containing 6, 7, and 8 N_{α} -methyltryptamine units were recently identified in another genus of Rubiaceae (9). The study of their cytotoxic properties on HTC cells could give additional results to verify our hypothesis.

Finally, it would be of interest to test these polyindoline alkaloids on other tumor cell lines as well as on normal cells *in vitro* and *in vivo*, in order to determine their acute and chronic toxicity on healthy animals.

References

- (1) Roth, A., Kuballa, B., Cabalion, P., Anton, R. (1985) Poster presented at "XXI^{èmes} Rencontres Internationales de Chimie Thérapeutique", Reims, Sept., 11-13.
- (2) Roth, A., Kuballa, B., Cabalion, P., Anton, R. (1985) *Planta Med.* 51, 289.
- (3) Libot, F. (1982) Thèse d'Université n° 263, Université de Paris XI.
- (4) Vercourdt, B. (1976) *Kew Bull.* 31, 181.
- (5) Creasey, W. A. (1975) in *The Catharanthus Alkaloids* (Taylor, W. I., Farnsworth, N. R., eds.), 209-236, Marcel Dekker, New York.
- (6) Scott, A. I. (1964) in: *Interpretation of the UV Spectra of Natural Products*. (Barton D. H. R., Doenig W., eds.) Pergamon Press, Vol. 7, p. 174, Oxford.
- (7) Thompson, E. B., Tomkins, G. M., Curran, J. F. (1966) *Proc. Natl. Acad. Sci. US*, 56, 296.
- (8) Saad, H., Kuballa, B., Anton, R. (1985) Poster presented at "XII^{èmes} Rencontres Internationales de Chimie Thérapeutique", Reims, Sept., 11-13.