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Alkaloids from Tabernaemontana psorocarpa

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Key Word Index:

Tabernaemontana psorocarpa; Apocynaceae; indole alkaloids; biosynthesis.

Abstract

The isolation of the alkaloids from two different samples of stem bark of *Tabernaemontana psorocarpa* is described. Both samples contained 16-epi-isositsirikine as the major alkaloid but differed from each other in the number and quantity of the other alkaloids. The following minor alkaloids were identified: 12-methoxy-14,15-dehydro-vincamine, vallesiachotamine, isovallesiachotamine, tetrahydroalstonine, coronaridine and voacangine.

Introduction

Although many *Tabernaemontana* species have been examined for their alkaloids, no research on the alkaloids of *T. psorocarpa* is known to the authors except for the remark of OLEITA that this species is suitable for the extraction of alkaloids [2]. In a previous publication we reported on the presence of the seco-iridoid-glucoside sweroside from *T. psorocarpa* [1]. In this paper the isolation and identification of the alkaloids from the stem bark is described.

Results and Discussion

Two different samples of *T. psorocarpa* collected in Ivory Coast in 1980 and 1981 respectively, have been investigated. Each sample consisted of leaves, twigs and stem bark. In a preliminary investigation only the stem bark was shown to contain alkaloids, therefore only this part has been investigated on a large scale. Using column chromatography one major alkaloid was isolated from the first bark sample and identified by means of its spectral data (UV, MS, ^{1}H-NMR, ^{13}C-NMR, $[\alpha]_D^{20}$ as either isositsirikine or 16-epi-isositsirikine. As it was not possible to determine the stereochemistry of C-16 unambiguously from the data published for isositsirikine [3] and 16-epi-isositsirikine [4], the two alkaloids have been prepared semi-synthetically from geissoschizine. Their ^{1}H-NMR spectra have been compared with the spectrum of the isolated compound. From the results obtained and by comparison with the original ^{1}H-NMR spectra recorded by KUTNEY [3] and GILBERT [4] it could be concluded that the isolated compound is 16-epi-isositsirikine (Fig. 1). This was later confirmed by data published by KAN et al. [5] which are in good agreement with the values presented in this paper. The C-16 stereochemistry of the isositsirikines has recently been revised [6]. The correct stereochemistry is presented in Fig. 1. Using preparative TLC also four minor alkaloids have been isolated from the first bark sample, and identified as 12-methoxy-14,15-dehydro vincamine (Fig. 2), a mixture of vallesiachotamine (Fig. 3), and isovallesiachotamine (Fig. 3), and

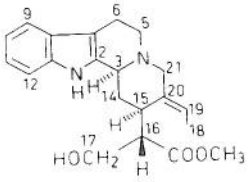

Fig. 1. 16-epi isositsirikine

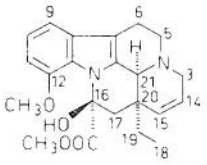

Fig. 2. 12-methoxy-14,15-dehydro vincamine

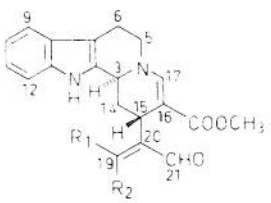

Fig. 3. Vallesiachotamine $R_1 = CH_3$, $R_2 = H$
Isovallesiachotamine $R_1 = H$, $R_2 = CH_3$

tetrahydroalstonine (Fig. 4) by means of their spectral data (UV, MS, ^{1}H-NMR). The identity of the last three compounds has been confirmed by coTLC with reference substances. In addition to the alkaloids – with the exception of tetrahydroalstonine – already found in the first sample, traces of a mixture of coronaridine (Fig. 5) and voacangine (Fig. 5) have also been isolated from the second bark sample and identified by means of coTLC in combination with ceric sulphate spray reagent.

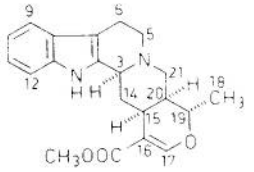

Fig. 4. Tetrahydroalstonine

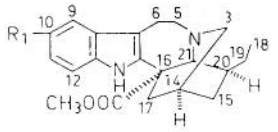

Fig. 5. Coronaridine $R_1 = H$
Voacangine $R_1 = OCH_3$

So far 16-epi-isositsirikine has been isolated from *Aspidosperma cuspa* [4] and *Tabernaemontana psychotrifolia* [7] (syn. *Peschiera echinata* [8]). 12-methoxy-14,15-dehydro-vincamine has been isolated from two species, namely *Crioceras dipladeniiflorus* [9] and *Hunteria elliotii* [10]. Alkaloids of the vincamine type are reported to occur in only two other *Tabernaemontana* species. Vallesiachotamine and isovallesiachotamine are rare alkaloids isolated for the first time by Djerassi from *Vallesia dichotoma* [11, 12]. So far they have always been isolated as a mixture. They have not been isolated before from the genus *Tabernaemontana*. Although tetrahydroalstonine has been isolated from many different genera, its occurrence in the genus *Tabernaemontana* is reported only once [13]. Coronaridine and voacangine occur in many *Tabernaemontana* species; in fact they are the most common alkaloids in the genus. The simultaneous occurrence of 16-epi-isositsirikine, vallesiachotamine and tetrahydroalstonine is of biosynthetic interest. All three alkaloids are examples of the early stages in the indole alkaloid biosynthesis. Both tetrahydroalstonine and 16-epi-isositsirikine probably have 4,21-dehydro-geissoschizine (Fig. 6) as precursor [14, 5]. The fact that only the 16S isomer of the isositsirikines has been isolated, shows that the enzym system in *T. psorocarpa* which is responsible for the reduction of the double bond in geissoschizine is stereo-specific. The enzym system present in *Aspidosperma cuspa*

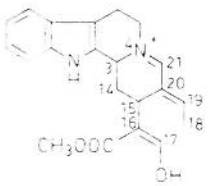

Fig. 6. 4,21-dehydro-geissoschizine

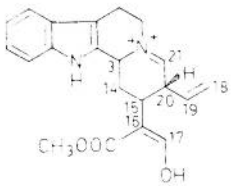

Fig. 7. 4,21-dehydro-corynantheine

and *T. psorocarpa* produces an isositsirikine with th opposite 16-configuration as the one in *Catharanth roseus* [3, 6]. Stöckigt has proposed that vallesiach tamine may be derived from 4,21-dehydro-coryna theine aldehyde (Fig. 7). 4,21-dehydro-corynanthe ne aldehyde hydrolyses via a carbinolamine to a dia dehyde. This is then converted, after isomerizatio of the 18,19 double bond, to a 19,20 double bond, b condensation between C_{17}-OH and N_4-H [14]. Poss bly 4,21-dehydrogeissoschizine, which is the prob ble precursor of both 16-epi-isositsirikine and tetra hydroalstonine, and not 4,21-dehydro-corynanthei aldehyde is the direct precursor of vallesiachotam ne. No isomerization would then be necessary. Th co-occurrence of tetrahydroalstonine and vallesi chotamine demonstrates that later enzymes of th heteroyohimbine pathway need not to be absent f the formation of alkaloids of the vallesiachotamin type, as suggested by Stöckigt [14]. The concurren production of the corynanthean, vallesiachotaman eburnan and ibogan classes thus makes *T. psoroca pa* a unique species from a biosynthetical viewpoi within the genus *Tabernaemontana*.

Experimental

Plant material

Two different samples, each consisting of stem bark, twigs a leaves of *T. psorocarpa* (Pierre ex Stapf) Pichon, were collecte by Dr. A. J. M. Leeuwenberg in Ivory Coast. The first sample, co lection no. Lg 12100, was collected early in 1980 10 km east Yakassé Mé. The second sample, collection no. Lg 12277, was co lected in late 1981 in Forêt de Yapo. Voucher specimens are ke in the herbarium at Wageningen, The Netherlands.

Isolation of the alkaloids

The isolation procedure and the quantities of stem bark use were the same for both samples. 225 g of the ground stem bark wa extracted for 12 hours with 96 % alcohol in a Soxlet apparatus wo king under reduced pressure (± 0.25 atm.). After filtration the a cohol was evaporated in vacuo to dryness. The extract (12 g) wa partitioned between 200 ml ethyl acetate and 200 ml 2 % ace

acid. The acetic acid layer was collected, brought to pH = 8 with dilute ammonium hydroxide and extracted with ethyl acetate. The ethyl acetate layer was collected, dried over anhydrous sodium sulphate and evaporated in vacuo to dryness. Yield: 110 mg of tertiary alkaloids (0.05 %). The extract was passed through a Merck Silica gel column, size B (dead volume of 90 ml) with ethyl acetate-isopropanol (1 + 1) as mobile phase. For both samples three fractions were collected. The second and third fraction of both samples contained pure 12-methoxy-14,15-dehydro-vincamine and 16-epi-isositsirikine respectively. In the first bark sample the first fraction was separated by means of preparative TLC on 0.25 mm silica gel PF254 layers with solvent system B in tetrahydroalstonine and a mixture of the vallesiachotamines. Although the two isomers can be separated by means of solvent E, this was not attempted due to the minimal quantity present. In the second bark sample fraction one was again separated with solvent B in two bands. The band with the lower R_f yielded again a mixture of the vallesiachotamines, while the band with the higher R_f was shown to be a mixture of coronaridine and voacangine by means of MS and NMR. This was confirmed by means of coTLC with solvent D. Coronaridine and voacangine were not further separated due to lack of material.

Chromatography

The TLC systems used were:
A: ethyl acetate – isopropanol 1 + 1
B: ethyl acetate – isopropanol 9 + 1
C: toluene – absolute ethanol saturated with ammonia 9 + 1
D: toluene – absolute ethanol saturated with ammonia 98 + 2
E: chloroform – cyclohexane – diethylamine 10 + 8 + 3.
All on silica gel F254 "fertig platte" Merck in saturated chambers.

Detection

The spray reagents used were:
A: 1 % ceric sulphate in 10 % sulphuric acid
B: 0.2M ferric chloride in 35 % perchloric acid, followed by heating with hot air
C: iodoplatinate reagent
D: Dragendorff's reagent.

Apparatus

UV spectra were recorded in methanol. 100 MHz ^{1}H-NMR and 25.2 MHz ^{13}C-NMR spectra were recorded on a JEOL PS-100 apparatus, 300 MHz ^{1}H-NMR spectra were recorded on a Bruker WM 300, both in the Fourier transform mode in $CDCl_3$. Shifts are presented in δ values relative to TMS. MS spectra were obtained with an AEI MS 902 spectrometer using a direct inlet system and ionization energy of 70 eV. $[\alpha]_D^{20}$ was recorded in $CHCl_3$.

Characterization of the alkaloids

16-Epi-isositsirikine: R_f-values in the TLC systems: A 0.19, B 0.07, C 0.31. Colour with spray reagent: A yellow, B green-black.
UV, λ_{max}: 223, 278 and 288 (sh) nm.
MS (160° C, 70 eV): m/z 355 (15), 354 (M^+, 67), 353 (54), 339 (3), 327 (21), 325 (4), 324 (7), 323 (24), 295 (6), 252 (33), 251 (100), 250 (50), 249 (37), 237 (16), 235 (9), 223 (14), 171 (27), 170 (32), 169 (22), 168 (18), 156 (23), 144 (19).
^{1}H-NMR (100 MHz): the ^{1}H-NMR spectrum was identical with the ^{1}H-NMR spectrum of synthesized 16-epi-isositsirikine and with the ^{1}H-NMR spectrum of 16-epi-isositsirikine isolated by Gilbert et al. [4].
^{13}C-NMR (25.2 MHz): 13.0 (q, C-18), 18.5 (t, C-6), 29.6 (t, C-14), 32.4 (d, C-15), 49.1 (d, C-16), 51.2 (t, C-5), 51.5 (q, $COOCH_3$), 52.5 (d, C-3), 54.6 (t, C-17), 61.6 (t, C-21), 107.3 (s, C-7), 111.0 (d, C-12), 118.0 (d, C-9), 119.2 (d, C-10), 121.4 (d, C-11), 122.5 (d, C-14), 127.2 (s, C-8), 133.5 (s, C-2), 134.5 (s, C-20), 136.2 (s, C-13), 174.1 (s, $COOCH_3$). $[\alpha]_D^{20}$: −97° (c = 0.19).

12-Methoxy-14,15-dehydro-vincamine: R_f-values in the TLC systems: B 0.40, C 0.69. Colour with spray reagent: A grey-black, B Pale blue-green.
UV, λ_{max}: 226, 271, 281 (sh), 294 nm.
MS (160° C, 70 eV): m/z 383 (15), 382 (M^+, 61), 381(11), 364 (4), 353 (22), 352 (11), 351 (11), 349 (8), 335 (12), 324 (27), 323 (100), 314 (5), 308 (12), 307 (8), 295 (7), 294 (9), 293 (8), 281 (4), 280 (54), 279 (27), 265 (11).
^{1}H-NMR (100 MHz): 7.08 (dd, J = 2.0 and 6.6 Hz, H-9), 7.08 (dd, J = 6.4 and 6.6 Hz, H-10), 6.58 (dd, J = 2.0 and 6.4 Hz, H-11), 5.78 (br d, J = 9.4 Hz, H-15), 5.48 (dt, J = 2.8 and 9.4 Hz, H-14), 4.26 (br s, OH), 4.04 (br s, H-21), 4.0–2.4 (m, H-5 and H-6), 3.85 (s, $COOCH_3$), 3.76 (s, OCH_3), 3.02 (m, H-3), 2.48 (d, J = 12.5 Hz, H-17), 2.10 (d, J = 12.5 Hz, H-17), 2.05 (dd, J = 7.6 and 12.5 Hz, H-19), 1.59 (dd, J = 7.6 and 12.5 Hz, H-19), 0.99 (t, J = 7.6 Hz, H-18).
Melting point: 208° C (uncorrected). $[\alpha]^{20}$: + 82° (c = 0.019).

Vallesiachotamine and isovallesiachotamine (mixture): R_f-values in TLC system: B 0.61, E 0.43 and 0.48. Colour with spray reagent: A red-brown, B orange-yellow.
UV, λ_{max}: 223 and 291 nm.
MS (150° C, 70 eV): m/z 351 (47), 350 (93), 335 (15), 323 (21), 322 (42), 319 (16), 318 (15), 317 (13), 307 (30), 291 (52), 279 (100), 265 (33), 264 (26), 263 (85), 221 (60), 209 (32).
^{1}H-NMR (100 MHz): 10.30 (s, H-21 isov.), 9.38 (s, H-21 vall.), 7.76 (s, H-17 isov.), 7.68 (s, H-17 vall.), 7.53–7.10 (m, H-9, H-10, H-11 and H-12), 6.67 (q, J = 6.5 Hz, H-19 vall.), 6.46 (q, J = 6.5 Hz, H-19 isov.),4.6–4.2 (m, H-3), 4.06–3.97 (m, H-15), 3.86–3.73 (m, H-6), 3.64 (s, $COOCH_3$), 2.94–2.83 (m, H-5), 2.18 (d, J = 6.5 Hz, H-18 isov.), 2.10 (d, J = 6.5 Hz, H-18 vall.), 2.15–1.7 (m, H-14). The ^{1}H-NMR data are in good agreement with those recently published by Waterman et al. [15].

Tetrahydroalstonine: R_f-values in TLC systems: B 0.67, C 0.72, D 0.39. Colour with spray reagent: A brown-black, B no colour.
UV, λ_{max}: 222, 282 and 289.
MS (125° C, 70 eV): m/z 352 (M^+, 100), 351 (55), 337 (28), 323 (6), 321 (5), 293 (7), 251 (17), 249 (11), 223 (23), 209 (11), 197 (18), 156 (43). ^{1}H-NMR (300 MHz): 7.78 (br s, NH), 7.56 (s, H-17), 7.45 (d, J = 6.7 Hz, H-9), 7.25–7.05 (m, H-10, H-11 and H-12), 4.50 (dd, J = 6.0 and 10.5 Hz, H-19), 3.75 (s, $COOCH_3$), 3.36 (dd, J = 2.5 and 11 Hz, H-3), 3.12 (dd, J = 2.5 and 13 Hz, H-21β), 3.0–2.85 (m, H5β and H6β), 2.82–2.65 (m, H6α, H-15 and H-21α), 2.6–2.45 (m, H-5α and H-14α), 1.40 (d, J = 6.2 Hz, H-18).

Coronaridine and voacangine (mixture): R_f-values in TLC systems: B 0.68, C 0.76, D 0.60 (cor.) and 0.52 (voa.). Colour with spray reagent: B coronaridine blue, purple after heating, voacangine purple, blue after heating.
UV, λ_{max}: 226, 283 and 294 nm.
MS (25° C, 70 eV): m/z 369 (10, voa.), 368 (M^+, 32, voa.), 353 (5, voa.), 339 (22, voa. and cor.), 338 (M^+, 74, cor.), 323 (12, cor.), 309 (3, voa. and cor.), 283 (2, voa.), 279 (2, cor.), 253 (5, cor.), 244 (6, voa.), 214 (15, cor.), 208 (13, voa. and cor.), 184 (12, voa.), 169 (20, cor.), 168 (8, cor.), 167 (11, cor.), 160 (7, voa.), 154 (19, voa. and cor.), 136 (100, voa. and cor.), 135 (36, voa. and cor.), 130 (10, cor.), 124 (50, voa. and cor.), 122 (39, voa. and cor.).
^{1}H-NMR (300 MHz): 7.77 (s, NH cor.), 7.64 (s, NH voa.), 7.48 (dd, J = 7.5 and 1 Hz, H-9 or H-12 cor.), 7.24 (dd, J = 7.5 and 1 Hz, H-9 or H-12 cor.), 7.15 (ddd J = 7.5, 7.5 and 1 Hz, H-10 or H-11 cor.), 7.13 (d, J = 9.0 Hz, H-12 voa.), 7.08 (ddd, J = 7.5, 7.5 and 1 Hz, H-10 or H-11 cor.), 6.92 (d, J = 2.5 Hz, H-9 voa.), 6.80 (dd, J = 9.0 and 2.5 Hz, H-11 voa.), 3.85 (s, OCH_3 voa.), 3.71 (s, $COOCH_3$ voa. and cor.), 3.85–1.30 (m, aliphatic protons), 0.90 (t, J = 7.4 Hz, H-18 voa. and cor.).

Synthesis of isositsirikine and 16-epi-isositsirikine

14 mg of geissoschizine was dissolved in 6 ml $C_2H_5OH - H_2O$ (5 + 1) and after addition of $NaBH_4$ stirred at room temperature for 1½ hours. The solution was then first acidified with 4 N HCl and subsequently basified with $KHCO_3$. The solution was then extracted twice with EtOAc after addition of 6 ml H_2O. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was separated by means of preparative TLC with solvent A in 2 bands. The lower, smaller band yielded a side product, which was shown by its MS and ^{1}H-NMR data to be most probably identical with isositsirikinediol. The higher, major band yielded 2 compounds in approximately equal quantities, which were separated

by means of preparative TLC with solvent E. The band with higher R_f was characterized as isositsirikine and the band with lower R_f as 16-epi-isositsirikine.

Isositsirikine: R_f-values in TLC systems: A 0.19, B 0.07, C 0.31, E 0.42. Colour with spray reagent: A yellow, B green-black.
MS (160° C, 70 eV): m/z 355 (17), 354 (M^+, 81), 353 (68), 339 (4), 337 (2), 325 (4), 324 (7), 323 (23), 295 (6), 252 (33), 251 (100), 250 (14), 249 (29), 237 (14), 235 (7), 223 (12), 171 (17), 170 (20), 169 (31), 168 (10), 156 (11), 144 (11).
^{1}H-NMR (300 MHz): 8.75 (br s, NH), 7.48 (d, J = 7.5 Hz, H-9), 7.39 (d, J = 8.1 Hz, H-12), 7.20–7.08 (m, H-10 and H-11), 5.64 (q, J = 6.8 Hz, H-19), 4.34 (br s, H-3), 3.82 (s, $COOCH_3$), 3.57 (dd, J = 7 and 11.6 Hz, H-17a), 3.56 (d, J = 12.5 Hz, H-21α), 3.50 (dd, J = 5.6 and 11.6 Hz, H-17b), 3.29 (br dd, J = 5.6 and 13.2 Hz, H-5β), 3.12–3.03 (m, 5α and H-15), 3.0 (m, H-6β), 2.97 (br d, J = 12.5 Hz, H-21β), 2.66 (br dd, J = 4.6 and 16 Hz, H-6α), 2.51 (m, H-16), 2.24 (m, H-14α and H-14β), 1.67 (dd, J = 1.0 and 6.8 Hz, H-18).

16-Epi-isositsirikine: R_f-values in TLC systems: A 0.19, B 0.07 and E 0.20. Colour with spray reagent: A yellow, B green-black.
MS (160° C, 70 eV): m/z 355 (17), 354 (M^+, 81), 353 (68), 339 (4), 337 (2), 325 (4), 324 (7), 323 (23), 295 (6), 252 (33), 251 (100), 250 (14), 249 (29), 237 (14), 235 (7), 223 (12), 171 (17), 170 (20), 169 (31), 168 (10), 156 (11), 144 (11).
^{1}H-NMR (300 MHz): 8.55 (br s, NH), 7.46 (d, J = 7.2 Hz, H-9, 7.34 (d, J = 7.8 Hz, H-12), 7.20–7.06 (m, H-10 and H-11), 5.53 (q, J = 6.7 Hz, H-19), 4.06 (br s, H-3), 3.95 (dd, J = 4.1 and 12 Hz, H-17a), 3.88 (dd, J = 5.0 and 12 Hz, H-17b), 3.80 (br d, J = 14.0 Hz, H-21β), 3.56 (s, $COOCH_3$), 3.33 (ddd, J = 4.7, 4.7 and 9.5 Hz, H-15), 3.20 (br dd, J = 5 and 12 Hz, H-5β), 3.12 (br d, J = 14.0 Hz, H-21α), 2.93 (m, H-6β), 2.85 (m, H-5α), 2.67 (br d, J = 15 Hz, H-6α), 2.60 (ddd, J = 4.1, 5.0 and 9.5 Hz, H-16), 2.30 (m, H-14α and H-14β), 1.61 (d, J = 6.7 Hz, H-18). The data presented here for the isositsirikines are in close agreement with the data published by Kan [5], except for the shift of the carbomethoxy group in isositsirikine, 3.82 and 3.67 ppm respectively.

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