SOME NEW DECUSSINE-TYPE ALKALOIDS FROM *STRYCHNOS DECUSSATA, STRYCHNOS DALE* AND *STRYCHNOS ELAECOCARPA*

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ABSTRACT.—From the stem bark of *Strychnos decussata* (Pappe) Gilg (Loganiaceae), four tertiary indole alkaloids have been isolated: bisnordihydrotoxiferine and three new alkaloids. The structures of two of the new alkaloids, 3,14-dihydrodecussine (**III**) and 10-hydroxy-3,14-dihydrodecussine (**IV**), have been ascribed on the basis of their spectral data. The muscle-relaxant activity of these new compounds is discussed.

Further studies of the alkaloids present in the stem bark of *Strychnos dale* and *Strychnos elaeocarpa* revealed the presence of decussine (**V**) and dihydrodecussine in *S. dale* and decussine, dihydrodecussine, and bisnordihydrotoxiferine in *S. elaeocarpa*.

During a pharmacological screening of some East African *Strychnos* species, it was found that the chloroform alkaloid fraction of *Strychnos decussata* had a strong muscle-relaxant effect (1). Subsequent research has been devoted to the isolation and establishment of the compounds responsible for this effect. In a series of previous publications, the isolation, identification, and pharmacological activity of a number of alkaloids from *S. decussata* were reported (2–5). In continuation of our studies of the alkaloids of *S. decussata*, four more tertiary indole alkaloids have been isolated, three new alkaloids related to decussine, a new alkaloid with muscle-relaxant properties (5), and previously known but new to this plant, bisnordihydrotoxiferine. Due to the relationship between the akagerine- and decussine-type alkaloids, the extracts of *S. dale* and *S. elaeocarpa*, both containing akagerine type of alkaloids, were further investigated for the presence of decussine-type alkaloids.

RESULTS AND DISCUSSION

Chemical

The structures of the alkaloids are given in fig. 1.

Strychnos decussata: Decussine (**V**), previously isolated from *S. decussata*, was obtained crystalline; structure **V** was confirmed by X-ray crystallography and its spectral data (5).

Decussine is a new pentacyclic indole alkaloid composed of a β-carboline skeleton possessing an azepino ring attached to a pyridine ring. Some of the decussine features are similar to the alkaloid camptoneurine, isolated from *S. camptoneura* by Koch *et al.* (6).

Two of the isolated alkaloids, **III** and **IV**, showed spectral data similar to that of decussine (**V**); all three were characterized by the presence of three nitrogens. The third nitrogen was, according to ^{1}H-nmr and ^{13}C-nmr, part of a pyridine nucleus. From the ^{1}H-nmr spectra, it was concluded that the pyridine nucleus of the alkaloids contained two ortho protons (a singlet, H–21 and a doublet, H–17) and a meta proton (doublet, H–16), i.e., the pyridine has its substituents

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in 3- or 4-position. The alkaloids contained one *N*-methyl group and one further methyl group each.

One of the new alkaloids, **III**, has a molecular weight of 303 (corresponding to $C_{20}H_{21}N_3$), i.e., two mass units higher than decussine. The ^{1}H-nmr spectra of **III** is similar to that of decussine (**V**) with the exception of the absence of the one proton singlet at δ 5.51, which indicates a saturation of the C3–C14 double bond in **III**. Furthermore, the singlet (143.90) and the doublet (95.04) in the aromatic region of the ^{13}C-nmr spectra of decussine (**V**) are replaced by a triplet (36.60) and a doublet (60.61) in the aliphatic region in the ^{13}C-nmr of alkaloid

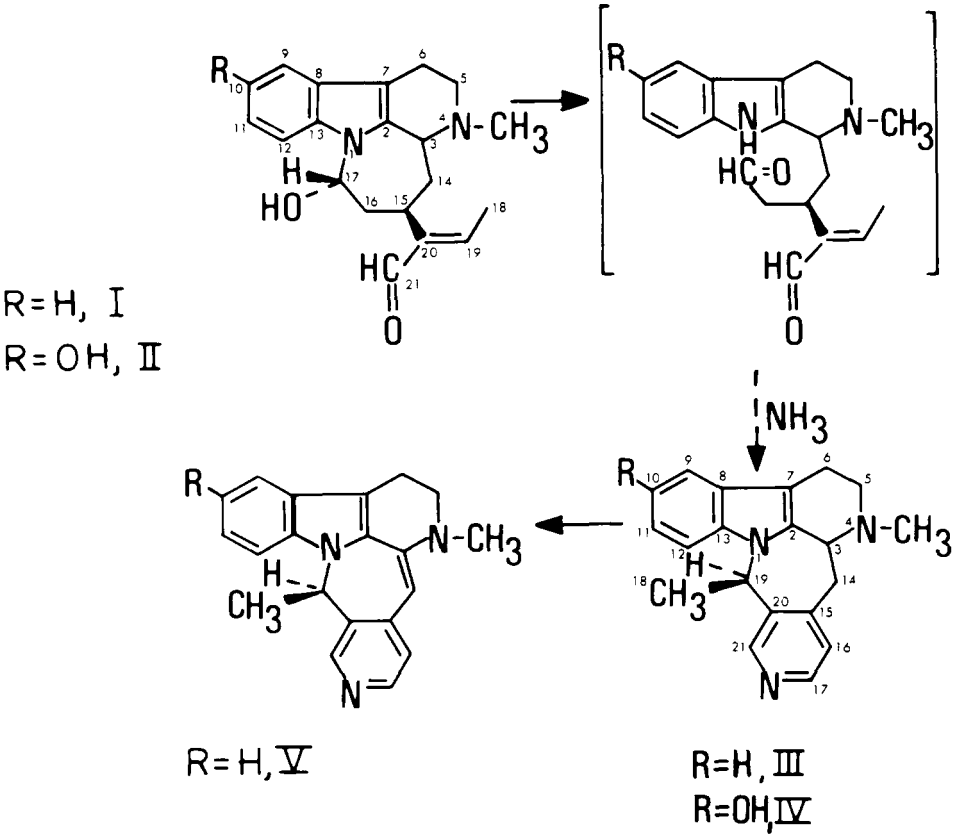

FIG. 1. Reaction sequence for the decussine-type alkaloids; **I** = akagerine, **II** = 10-hydroxyakagerine, **III** = 3,14-dihydrodecussine, **IV** = 10-hydroxy-3,14-dihydrodecussine, **V** = decussine.

III. From these data it is concluded that the new alkaloid is 3,14-dihydrodecussine (**III**). Reduction of decussine with $LiAlH_4$ yielded a minute amount of dihydrodecussine, which was identified by means of tlc. Further evidence for the relationship between **III** and **V** is the ready conversion of **III** to **V** under normal storage conditions.

3,14-Dihydrodecussine (**III**) and alkaloid **IV** showed very similar spectral data. The ms of **IV** showed a molecular ion at *m/z* 319 and a constant difference of 16 mass units for the fragments containing the aromatic moiety compared to **III**. Alkali-induced bathochromic shift in the uv spectrum and the presence of an ir absorption peak at 3380 cm^{-1} suggested the presence of a phenolic group in **IV**. The aromatic region of the ^{1}H-nmr spectra of **IV** showed a characteristic splitting pattern of a 10- or 11-hydroxy-substituted alkaloid as shown for 10-

hydroxyakagerine and -kribine types of alkaloids already isolated from *S. decussata* (3, 4). On the basis of the spectral data and taking into account previously isolated 10-hydroxy-derivatives, this alkaloid is suggested to be 10-hydroxy-3,14-dihydrodecussine (**IV**).

The spectral data of the third new alkaloid, named rouhamine, showed some similarities with decussine (**V**). The molecular weight of the new alkaloid is 299, two mass units lower than decussine. Decussine composes rapidly into this new alkaloid which, has an intense yellow color in day light and a yellow fluorescence (in 366 nm). This alkaloid was also isolated from *S. decussata*, and the structure of it is still under investigation.

The decussine-type alkaloids containing a third nitrogen might be artifacts due to the reaction of either ammonia used during extraction or to nitrogen-containing substances in the plant material taking over the function of ammonia under basic conditions. Similar reactions are known for the iridoids loganine and swertiamarine leading to the alkaloids gentianine and cantleyine, both containing a pyridine function (7). Another example is the angustine type of alkaloids (8).

The decussine-type alkaloids could be formed by the reaction sequence as given in scheme 1. This scheme would apply for artifact formation as well as biosynthesis. As precursors of the decussine-type alkaloids, akagerine-type alkaloids earlier isolated from *S. decussata* can be postulated. Exposure of akagerine-type alkaloids to ammonia in ethanol solution did not give any clear results. Tlc showed faint spots which might be decussine and dihydrodecussine. However, most of the alkaloids were unchanged. On the other hand, extracts from an extraction method avoiding the use of ammonia did not show any detectable amounts of either decussine or dihydrodecussine. Further studies of the origin of decussine-type alkaloids are desirable but could not be performed because of lack of material.

From a toluene extract of *S. decussata*, the main alkaloid was isolated and identified as bisnordihydrotoxiferine by means of uv, ir and ms and by comparison with an authentic sample (tlc).

Strychnos dale and Strychnos elaeocarpa: Because of the presence of akagerine-type alkaloids occurring both in *S. dale* and *S. elaeocarpa* (9), the extracts of these two species were further investigated for the presence of decussine-type alkaloids.

In the tertiary alkaloid fraction of *S. dale*, decussine and dihydrodecussine were identified by means of tlc. From fractions 3 and 4 [table III, p. 271 (9)] obtained from column chromatography separation of the tertiary alkaloids, alkaloid D_4 was isolated and identified as dihydrodecussine by means of tlc, uv, ir, ms, ^{1}H-nmr and ^{13}C-nmr. From column chromatography separation of the tertiary alkaloids of *S. elaeocarpa*, alkaloid E_4 was isolated from fractions 5 and 6 [table III, p. 271 (9)] and identified by means of tlc, uv, ir, ms, ^{1}H-nmr as dihydrodecussine. From one of the less polar fractions of *S. elaeocarpa*, decussine was isolated by means of preparative tlc and identified by means of uv, ir and ms. Furthermore, a basic toluene extract was made of a small amount of *S. elaeocarpa*. The main alkaloid of this extract was isolated and identified as bisnordihydrotoxiferine (tlc, uv, ir, ms).

Chemo-taxonomy

It is interesting to note that the four species, *S. decussata*, *S. dale*, *S. elaeocarpa* (9), *S. floribunda* (10), from which akagerine- and decussine-type alkaloids have been isolated, all belong to the same section Rouhamon of the genus Strychnos. *S.*

usambarensis in the same section has also been shown to contain akagerine (11). Bisnordihydrotoxifreine has also been reported to be present in four species belonging to the section *Rouhamon* [*S. variabilis* (12), *S. decussata*, *S. elaeocarpa* and *S. floribunda* (10)]. Furthermore, it has been found in three species belonging to the section *Strychnos* [*S. toxifera* (13), *S. pseudoquina* (14), *S. amazonica* (15)] and in four species belonging to the section Breviflorae [*S. dolichothyrsa* (16), *S. urceolata* (17)], *S. afzelli* (19) and *S. icaja* (19)].

Of seven African species of the section *Rouhamon* which have been studied, five have now been shown to contain very similar alkaloids, giving phytochemical support for the placing of these species into the section *Rouhamon*.

Pharmacology

In a previous publication (5), decussine was reported to have a pronounced muscle-relaxant activity, both *in vivo* and *in vitro*. A dose of 50 mg/kg gave a graded response of XX in the screen grip test (20). A concentration of 5.1 μg/ml (16 nmole/ml) of decussine reduced the amplitude of the contractions of a rat diaphragm, elicited by electrical stimulation of the phrenic nerve, to 50%; the effect was not antagonized by synstigmine (Neostigmine®).

Due to the great similarities between the structures of **III** and **IV** and the muscle-relaxant decussine (**V**), it was considered of interest to test whether the new alkaloids also had a muscle-relaxant effect. Unfortunately, 10-hydroxy-3,14-dihydrodecussine (**IV**) could not be tested due to a lack of material and a limited amount of rouhamine, made only in *vitro* diaphragm preparation possible. Rouhamine had a pronounced muscle-relaxant activity, giving a 50% inhibition of the amplitude of the contractions at a dose of 16 μg/ml, which was not antagonized by synstigmine (Neostigmine®).

When 3,14-dihydrodecussine (**III**) was tested on the rat diaphragm preparation, it did not show any effect on the amplitude of the contractions at a dose as high as 50 μg/ml (167 nmoles/ml). In the *in vivo* screen grip test, a dose of 225 mg/kg of 3,14-dihydrodecussine (**III**) gave a weak muscle-relaxant effect [graded response of X (20)].

The pharmacological results of decussine and dihydrodecussine points to a possible role of the double bond between C3 and C14 for the muscle relaxant effect.

EXPERIMENTAL[4]

PLANT MATERIAL.—The stem bark of *Strychnos decussata* (Pappe) Gilg, collection number Lg 10797, was collected in Karawa, Kenya, in November 1975. The stem bark of *Strychnos dale* De Wild and *Strychnos elaeocarpa* Gilg ex Leeuwenberg, collection numbers 7877 and 7860, respectively, were collected during June-July 1970 in Kribi, Cameroun, and another example of *S. elaeocarpa*, Lg. 10586, was collected in Western Cameroun in 1972. The three species were identified by Dr. A.J.M. Leeuwenberg at the Herbarium at Wageningen, The Netherlands, where voucher specimens are kept as references.

EXTRACTION OF THE ALKALOIDS.

Acid extraction: The method used to give the acid extract, from which the alkaloids of decussine type were isolated, was described earlier (4).

Toluene extraction: A small amount of plant material, basified with 10% $NaHCO_3$, was extracted with toluene. The toluene extract was then extracted with 1% acetic acid, basified with 10% $NaHCO_3$, and extracted again with toluene.

[4]All ^{1}H-nmr spectra were obtained in $CDCl_3$ with a Jeol 100 MHz instrument, while the ^{13}C-nmr spectra were recorded on a Varian 100 MHz instrument. Mass spectral analysis was carried out with a LKB 9000 instrument at 70 eV with direct inlet. The melting point was determined with a Leitz Mikroskopheiztisch 350. The ir spectrum was obtained using a Jasco-IRA-1 spectrophotometer (KBr-discs), while the uv spectrum was recorded in ethanol solution on a Schimadzu MPS-5000 UV-VIS spectrophotometer.

ISOLATION OF THE ALKALOIDS.—The chloroform fraction (19 g) from the acid extraction was chromatographed over silica gel and eluted, first, with chloroform and, subsequently, with increasing concentration of methanol in chloroform, up to 30% methanol. Fractions (20 ml) were collected and grouped according to the results of the tlc check. From different fractions, the three new alkaloids were obtained pure with the aid of preparative tlc.

From the toluene extract, bisnordihydrotoxiferine was isolated by means of preparative tlc.

Silica gel 60 (70–230 mesh, E. Merck) was used for column chromatography. Thin-layer chromatography was carried out on either precoated silica gel 60 plates (0.25 mm, silica F_{254}, E. Merck) or on a 0.5 mm layer of silica gel GF_{254} (60, E. Merck) spread on 20 x 20 cm glass plates with the following solvent systems:

A. Chloroform-methanol (9:1)
B. Cyclohexane-chloroform-diethylamine (6:3:1)
C. Diethyl ether-ethanol-diethylamine (90:3:7)

The tlc plates were developed in saturated cha bers, and the colors of the alkaloids were registered after spraying with: 1% ceric sulfate in 1 M sulfuric acid or 0.2 M ferric chloride in 35% perchloric acid, followed by heating for 2–5 min. in a stream of hot air.

PHYSICAL DATA OF THE ALKALOIDS.—For ^{13}C-nmr see table 1.

Decussine (110 mg) (**V**) was purified by preparative tlc with diethyl ether-ethanol-diethylamine (90:3:7) as mobile phase. It crystallized as yellow prisms from methanol, mp 203–205°; uv: λ max (log ε): 225 (4.42), 254 (4.30), 260 (sh, 4.28), 304 (4.20), 319 (4.21), 362 (4.32) and 390 (4.39) nm; ir: ν max: 3040, 3000, 2830, 1595, 1540, 1490, 1465, 1420, 1340, 1205, 1090, 1070, 1030, 980, 860 and 760 cm^{-1}; ms: *m/z* (%): 301 (52, M$^+$), 287 (23), 286 (100), 271 (4), 270 (12), 269 (4), 257 (5), 256 (5), 244 (5), 243 (7), 242 (6), 213 (6), 151 (9), and 143 (9); ^{1}H-nmr: δ8.37 (s, 1H, H-21), 8.27 (d, 1H, *J*=5Hz, H-17), 7.63–7.03 (m, 4H, H-9, H-10, H-11, H-12), 6.96 (d, 1H, *J*=5Hz, H-16), 5.70 (q, 1H, *J*=7Hz, H-19), 5.51 (s, 1H, H-14) 3.15 (s, N-CH$_3$), 1.43 (d, 3H, *J*=7Hz, H-18 ppm. Rf-values in tlc systems A, B and C were 0.48, 0.53 and 0.57, respectively. The alkaloid was detected by its intense blue green to yellow green fluorescence (366 nm) and by its brown color both with ceric sulfate and ferric chloride spray reagent.

3,14-Dihydrodecussine (15 mg) (**III**) was purified by preparative tlc with cyclohexane-chloroform-diethylamine (6:3:1) as mobile phase and was obtained as brown crystals from ethanol: mp 78–82°; uv: λ max (log ε): 230 (4.00), 268 (3.62), 279 (3.64), 2.84 (3.66) and 295 (3.63) nm; ir: ν max: 2990, 2940, 2840, 2790, 1595, 1460, 1455, 1320, 1190, 1165, 1050, 820 and 740 cm^{-1}; ms: *m/z* (%): 303 (57, M$^-$), 302 (29), 288 (23), 261 (12), 260 (45), 259 (7), 246 (11), 245 (48), 185 (26), 184 (100), 183 (64), 182 (11), 144 (6), 143 (9), 142 (11) and 130 (9); ^{1}H-nmr: δ 8.61 (s, 1H,

TABLE 1. ^{13}C-nmr. Chemical shifts of decussine (**V**) and 3,14-dihydrodecussine (**III**).

Carbon	V	III
C-2	130.05[a]	133.60[a]
C-3	143.90[b]	60.61
C-5	52.06	53.00
C-6	21.49	18.61
C-7	114.20	108.02
C-8	125.90	126.75
C-9	119.20	118.30
C-10	123.07[c]	121.67
C-11	120.10	119.52
C-12	109.68	109.45
C-13	137.30	136.24
C-14	95.04	36.60
C-15	144.60[b]	146.95
C-16	123.40[c]	125.01
C-17	147.64[d]	149.25[b]
C-18	18.99	22.64
C-19	53.99	55.09
C-20	129.40[a]	134.61[a]
C-21	147.93[d]	150.01[b]
N-CH$_3$	40.32	37.26

[a, b, c, d]Assignments of signals with identical superscripts in each column may be interchanged.

H-21), 8.54 (d, 1H, J=5Hz, H-17), 7.60-7.18 (m, 4H, H-9, H-10, H-11, H-12), 7.10 (d, 1H, J=5Hz, H-16), 5.78 (q, 1H, J=H7z, H-19), 2.53 (s, N-CH_3), 1.66 (d, 3H, J=7Hz, H-18) ppm. Rf-values in tlc-systems A, B and C were 0.24, 0.40 and 0.34, respectively. Dihydrodecussine gave a yellowish brown color both in ceric sulfate spray reagents and ferric chloride reagents.

10-Hydroxy-3,14-dihydrodecussine (7 mg) (**IV**) was purified by preparative tlc with diethyl ether-ethanol-diethylamine (90:3:7) as mobile phase. 10-Hydroxy-3,14-dihydrodecussine was obtained as pale brown solid; uv: λ max (log ε): 230 (4.20), 263 (3.88), 270 (3.91), 282 (3.92), 309 (3.75) and 320 (sh, 3.61) nm; by addition of KOH: λ max (log ε): 282 (3.40) and 334 (3.04) nm; ir: ν max: 3380, 2950, 2790, 1600, 1585, 1460, 1390, 1345, 1210, 1165, 1056, 940, 850 and 780 cm^{-1}; ms: *m/z* (%): 319 (96, M^+), 318 (52), 317 (28), 304 (37), 303 (16), 302 (57), 290 (7), 286 (7), 277 (17), 276 (75), 275 (12), 261 (58), 201 (16), 200 (100), 190 (70), 185 (7), 184 (5), 144 (5), 143 (5), and 130 (8); ^{1}H-nmr: 8.58 (s, 1H, H-21), 8.50 (d, 1H, J=5Hz, H-17), 7.25 (d, 1H, J=9Hz, H-12), 6.89 (d, 1H, J=2Hz, H-9), 6.80 (dd, 1H, J=9Hz, J=2Hz, H-11), 5.72 (q, 1H, J=7Hz, H-19), 2.53 (s, N-CH_3) and 1.59 (d, 3H, J=7Hz, H-18) ppm.

Rouhamine (16.7 mg) was purified by preparative tlc with diethyl ether-ethanol-diethylamine (90:3:7) as mobile phase. Rouhamine crystallized from methanol/diethylether as yellow needles, mp 205–210°; uv: λ max (log ε): 227 (4.36), 257 (4.03) and 310 (3.93) nm; ir: ν max: 2960, 2820, 2760, 2470, 1660, 1620, 1470, 1450, 1430, 1390, 1340, 1280, 1210, 1150, 1130, 1050, 960, 800 and 755 cm^{-1}; ms: *m/z* (%): 299 (45, M^+), 298 (3), 285 (22), 284 (100), 270 (7), 256 (27), 229 (5), 184 (4) and 140 (5). R_f-values in the tlc systems A, B and C were: 0.58, 0.45 and 0.30, respectively. Rouhamine gave a red color with ceric sulfate reagent and a brown color with ferric chloride reagent.

Bisnordihydrotoxiferine was purified by preparative tlc with cyclohexane-chloroform-diethylamine (6:3:1) as mobile phase. The alkaloid gave a violet color in ceric sulfate immediately after spraying.

REDUCTION OF DECUSSINE.—Decussine was dissolved in diethylether and a diethylether suspension of lithiumaluminiumhydride was gradually added. The mixture was left to stand at room temperature for 48 h and was then refluxed for 5 h. 3,14-Dihydrodecussine was detected on tlc. The amount of 3,14-dihydrodecussine was too small to isolate. The tlc systems used to detect 3,14-dihydrodecussine were systems A and B; the alkaloid gave a yellowish brown color both in ceric sulfate and ferric chloride spray reagents.

PHARMACOLOGICAL.—The screen grip test was performed on female mice of the NMRI strain weighing 18–20 g. Dihydrodecussine was dissolved in saline (0.9% NaCl). An equivalent amount of citric acid was added, and the mixture was injected intraperitoneally. There were a total of five mice on three different doses (20). In order to establish the muscle-relaxant effect, the rat diaphragm preparation was used (21). Krebs' solution was used instead of Tyrode's solution. The dose of rouhamine (four deteminations on two different does) which gave 50% inhibition of the amplitude of the muscle response was calculated by plotting the percentage of response in probit of the muscular contraction versus log dose (22).

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LITERATURE CITED

1. M. Geevaratne, W. Rolfsen and L. Bohlin, *Acta Pharm. Suec.*, **14**, 43 (1977).
2. A. Petitjean, P. Rasoanaivo and J. H. Razafintsalama, *Phytochemistry*, **16**, 154 (1977).
3. W. N. A. Rolfsen, A. A. Olaniyi and P. J. Hylands, *J. Nat. Prod. (Lloydia)*, (in press).
4. A. A. Olaniyi and W. N. A. Rolfsen, *J. Nat. Prod. (Lloydia)*, (in press).
5. W. N. A. Rolfsen, A. A. Olaniyi, F. Sandberg and Å. H. Kvick, *Acta Pharm. Suec.*, (in press).
6. M. Koch, J. Garnier and M. Plat, *Ann. Pharm. Franc.*, **30**, 299 (1972).
7. N. G. Bisset, *Pharm. Weekbl.*, **110**, 425 (1975).
8. J. D. Phillipson, S. R. Hemingway, N. G. Bisset, P. J. Houghton and E. J. Shellard, *Phytochemistry*, **13**, 973 (1974).
9. W. Rolfsen, L. Bohlin, S. K. Yeboah, M. Geevaratne and R. Verpoorte, *Planta Med.*, **34**, 264 (1978).
10. Unpublished results, R. Verpoorte.

11. L. Angenot, O. Dideberg and L. Dupont, *Tetrahedron Lett.*, **16**, 1357 (1975).
12. M. J. G. Tits and L. Angenot, *Planta Med.*, **34**, 57 (1978).
13. H. Asmis, P. Waser, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **38**, 1661 (1955).
14. F. Delle Monache, P. T. Aldo and G. B. Marini-Bettòlo, *Tetrahedron Lett.*, **25**, 2009 (1969).
15. G. B. Marini-Bettòlo and F. Delle Monache, *Gazz. Chim. Ital.*, **103**, 543 (1973).
16. R. Verpoorte and A. Baerheim Svendsen, *Lloydia*, **39**, 357 (1976).
17. R. Verpoorte, E. W. Kodde and A. Baerheim Svendsen, *Planta Med.*, **34**, 62 (1978).
18. R. Verpoorte, E. W. Kodde, H. Van Doorne and A. Baerheim Svendsen, *Planta Med.*, **33**, 237 (1978).
19. K. Kambu, C. Coune and L. Angenot, *Planta Med.*, **37**, 161 (1979).
20. F. Sandberg, R. Verpoorte and A. Cronlund, *Acta Pharm. Suec.*, **8**, 341 (1971).
21. Pharmacological Experiments On Isolated Preparations. By staff of the Dept. of Pharmacology, Edinburgh University, pp. 30–37 Livingstone (1966).
22. D. J. Finney. *Probit Analysis*. Cambridge University Press, 203 (1974).