PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON SOME ALKALOIDAL PLANTS OF EGYPT

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It was found on checking literature that several plants of the Egyptian flora are more or less unknown from the phytochemical points of view. In collaboration with Prof. Vivi Tackholm, University of Cairo, and Mr. Mohammed Drar, former Keeper of the Herbarium, Agricultural Museum, Cairo, a list was drawn up of those species which might be of medical interest on a systematic basis. One of these species is *Retama Raetam* Webb & Berth. (*Genista Raetam* Forsk.) of the Leguminosae family, which has not been investigated previously.

On systematic grounds, the presence of lupine alkaloids could be expected. This assumption was confirmed. The two main alkaloids (Fig.1) found

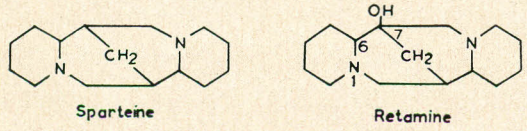


Fig. 1.—Structure of sparteine and retamine.

in the tops of the plant and *d*-sparteine (0.70-0.81 per cent) and retamine (0.21-0.25 per cent). Traces of five other hitherto unidentified alkaloids were detected by paper chromatography.

Plant material collected in August and March showed the same quantitative and qualitative alkaloid content. The total amount of alkaloids in the fruits was 0.14 per cent, consisting of the same alkaloids as those occurring in the tops of the plants (stem and leaves).

d-Sparteine has been isolated from several species of the Leguminosae family: *Ammodendron conollyl* Bge., *Ammothamnus lehmanni* Bge., *Anagyris foetida* L., *Baptisia australis* (L). R. Br., *B. minor* Lehm., *B. perifoliat* (1) *R. Br.*, *Cytisus caucasicus* Hort., *Lupinus pusillus* Pursh, *Genista sphaerocarpa* Lam., *Sophora pachycarpa* C.A. Mey., *Thermopsis lanceolata R. Br.*

Retamine, a hydroxy sparteine, has hitherto been found only in two other species. It has been isolated from *Genista sphaerocarpa* Lam. (*Retama sphaerocarpa* Boiss.) by Battandier and Malosse [1] Wunschendorff and Valier[2] and Ribas et al.,[3] and from *Genista aethnenis* DC. by White.[4]

According to Ribas and Fraga[5] the probable position of the OH group is not, as suggested previously, at C_6 but rather at C_7 (cf. the formula).

The procedure for isolating the alkaloids from the powdered plant material was as follow . The acidic ethanol extract was evaporated, the remaining water solution made alkaline, and extracted with ether. Retamine crystallized from the dried solution. The ether was evaporated completely, and the liquid residue distilled with the aid of steam. *d*-Sparteine was isolated from the steam-volatile fraction. The minute quantity of non-volatile residue after distillation was dissolved in hydrochloric acid and separated chromatographically on a paper-powder column.

The solvent used is a slight modification of that described by Munier et al:[6] *n*-butanol, concentrated hydrochloric acid and water (added in portions until saturation) (98:2:21). Afterwards, 5 ml. of *n*-butanol was added.

The atmosphere of the chromatography tank was saturated with the vapour from one beaker containing water and one containing *n*-butanol and hydrochloric acid (98:2).

The chromatograms were run at a temperature of 20° ±1 C. The paper (Whatman no. 1) was impregnated with 0.5 M KCl, dried at 110°C. The colour reagent for detection of the alkaloids is a modification of the Dragendorff reagent described by Block, Durrum and Zweig.[7] The chromatogram (no. 4 in Fig. 2) shows the presence of five minor alkaloids with the following R_f values: 0.034, 0.11, 0.20, 0 51 and 0.73.

The melting points of the picrates and their corresponding Rf values were as follows: 185 ·210°C for the alkaloid with the Rf value 0.11; 170-180°C (R^f=0.20); 140-160°C. (R_f 0.51) and 70°C. (R^f=0.73).

Owing to the minute quantities available, no further analysis of these minor alkaloids was possible.

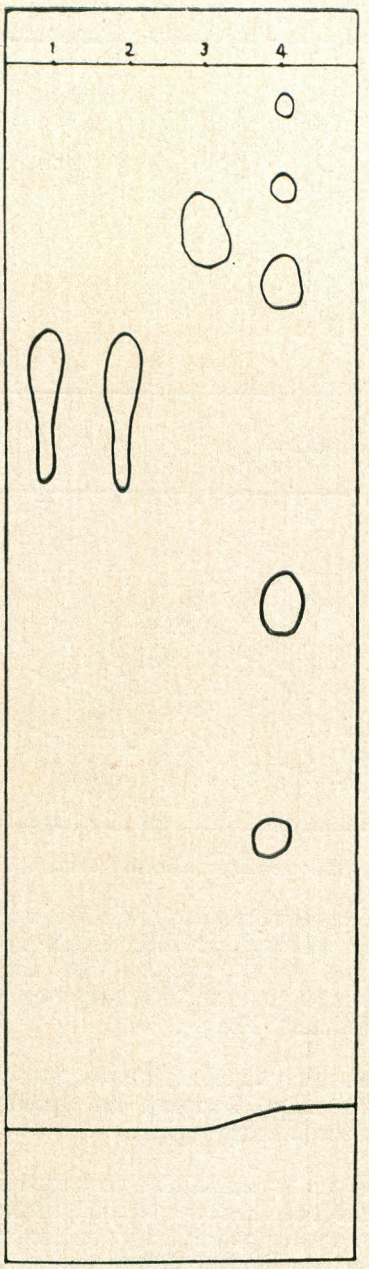


Fig. 2.—Paper chromatogram of l-sparteine sulphate Merck(1), d-sparteine sulphate (2), retamine dihydro-chloride (3), and minor alkaloids (4).

The identification of *d*-sparteine and retamine was based on elementary analysis, melting points, optical activity, paper chromatography and infrared spectra (Figs. 3 and 4).

d-Sparteine and retamine are rather unknown, from pharmacological points of view, whereas the *l*-isomer of sparteine, *l*-sparteine has long been used as an oxytocic agent.

A comparative study of the effects of these three structurally related alkaloids d-,l-sparteine, retamine on the spontaneous in vitro motility in the corpus and isthmus of the pregnant and non-pregnant human uterus has recently been performed.[8]

The results were statistically analyzed considering the following parameters:

1. Incidence of response
2. Change of motility pattern
3. Tonus
4. Frequency
5. Amplitude
6. Amplitude maximum

The estimation was made as follows:

1. *Incidence of Response.*—The number of responses (reactors) as percentage of the total number of doses administered (reactors and non-reactors).

The non-reactors were excluded and the following factors are calculated as percentages of the reactors.

2. *Change of Motility Pattern.*—Change from the type of motility to another following the addition of drug.

3. *Tonus.*—Variation of base line (a line drawn through the amplitude minima). The positive variation was estimated as +, ++, and +++, corresponding to an increase in tonus of less than 50 per cent, 50-100 per cent and more than 100 per cent of the height of the original amplitude, respectively.

The negative variation was defined in a corresponding manner.

4. *Frequency.*—Variation in number of contractions er minute. The positive variation was estimated as +, ++, and +++, corresponding to an increase of less than 100 per cent, 100—200 per cent, more than 200 per cent of the original frequency of contractions, respectively.

The negative variation was defined in a corresponding manner.

5. *Amplitude.*—Variation in height of the contractions. The positive variation was estimated as +, ++, and +++, corresponding to an increase in top line of less than 50 per cent, 50-100 per cent and more than 100 per cent of the original amplitude, respectively.

The negative variation was defined in a corres-

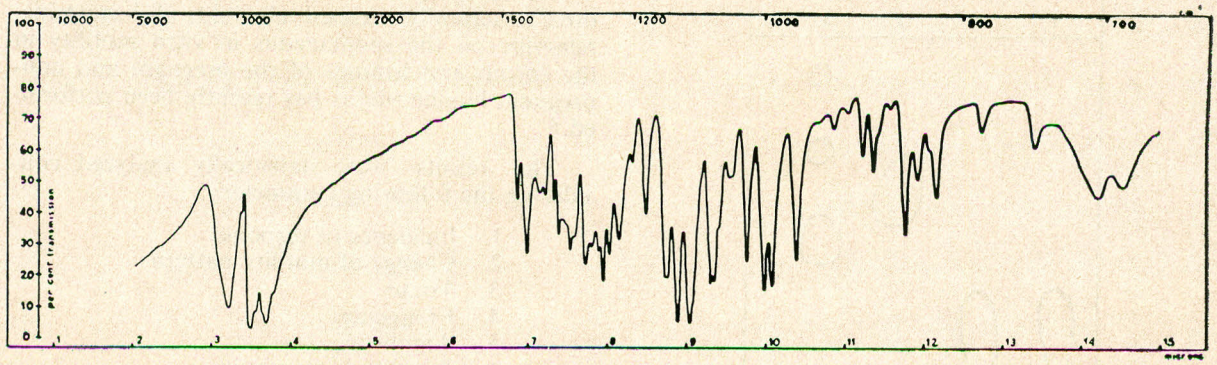


Fig. 3.—Infrared absorption spectrum of retamine.

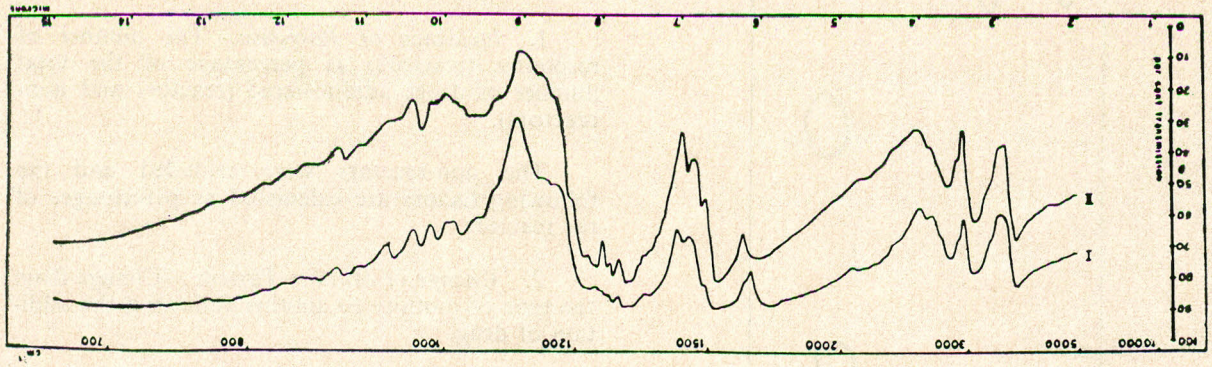


Fig. 4.—Infrared spectra of d-sparteine sulphate (curve I) and l-sparteine sulphate Merck (curve II).

ponding manner.

6. *Amplitude Maximum.*—Variation of top line (a line drawn through the amplitude maxima). The positive variation was estimated as +, ++, and +++, corresponding to an increase in top line of less than 50 per cent, 50-100 per cent and more than 100 per cent of the original amplitude.

The negative variation was defined in a corresponding manner.

l-Sparteine (Table 1) gives an oxytocic action as a whole, the degree of which varies in different parts of the uterus and during the sexual phases, being most pronounced in the lower segment of the pregnant uterus (Fig. 5).

d-Sparteine (Table 2) exerted an oxytocic action on the isthmus and corpus of both pregnant and non-pregnant uterus. A comparison between the effects of the optical isomers of sparteine shows great similarities. An evident difference is observed in the corpus, where frequency is increased by *d*-sparteine but unchanged by *l*-sparteine both in the pregnant and non-pregnant organ.

The effects of retamine (Table 3) is very interesting. It has an oxytocic effect on the whole pregnant human uterus and on the non-pregnant corpus, but acts spasmolytically on the non-pregnant isthmus (Fig. 6).

This spasmolytic effect is most pronounced on the muscle strips, showing the specific motility pattern of the isthmus region.

It is especially remarkable that retamine has an oxytocic effect on the lower segment of the pregnant uterus, but acts spasmolytically on the corresponding part of the non-pregnant organ. The effect described is not abolished by atropinisation or ganglionic blockade. This indicates that the qualitatively altered reactivity of the muscle cells of the isthmus region in pregnancy at term is confined to the muscle cell per se. However, at present the biochemical explanation for this interesting finding cannot yet be given.

It was mentioned that there are different motility patterns in the corpus and in the isthmus region. Quantitatively different pharmacological response of the musculature from the corpus and

TABLE 1.—THE EFFECTS OF L-SPARTEINE ON HUMAN UTERUS IN VITRO TESTED ON 30 NON-PREGNANT AND 10 PREGNANT UTERI.

Parameter	Non- regnant uterus				Pregnant uterus	
	Corpus		Isthmus			
	Prolif.	Secr.	Prolif.	Secr.	Corpus	Lower segment
Incidence of response ..	21/26	43/56	28/35	37/41	22/35	41/50
..	80%	77%	80%	91%	63%	82%
Change of motility pattern % ..	48	29	33	41	18	27
Tonus increase % ..	75	51	59	22	36	44
Tonus unchanged %	19	29	37	70	50	56
Tonus decrease % ..	6	20	4	8	14	0
Frequency increase % ..	17	25	70	49	23	78
Frequency unchanged % ..	67	44	26	27	50	15
Frequency decrease % ..	16	31	4	24	27	7
Amplitude increase % ..	33	32	26	65	46	61
Amplitude unchanged %	24	23	30	22	36	34
Amplitude decrease % ..	43	45	44	13	18	5
Amp max. increase % ..	76	35	30	65	55	71
Amp max. unchanged % ..	24	56	66	30	1	29
Amp max. decrease % ..	0	9	4	5	14	0

TABLE 2.—THE EFFECTS OF *d*-SPARTEINE ON HUMAN UTERUS IN VITRO ESTED ON 26 NON-PREGNANT AND 18 PREGNANT UTERI.

Parameter	Non-pregnant uterus				Pergnant uterus	
	Corpus		Isthmus			
	Prolif.	Secr.	Prolif.	Secr.	Corpus	Lower segment
Incidence of response ..	36/39	30/33	30/34	52/60	40/49	36/42
..	92%	91%	88%	87%	82%	86%
Change of motility pattern % ..	25	33	13	31	13	19
Tonus increase % ..	67	70	43	44	58	67
Tonus unchanged % ..	28	30	53	46	40	33
Tonus decrease % ..	5	0	4	10	2	0
Frequency increase % ..	61	44	60	69	63	70
Frequency unchanged % ..	17	33	27	14	15	22
Frequency decrease % ..	22	23	13	17	22	8
Amplitude increase % ..	31	37	40	38	17	44
Amplitude unchanged % ..	22	37	37	46	50	39
Amplitude decrease % ..	47	26	23	16	33	17
Ampl. max. increase % ..	39	77	57	62	47	75
Ampl. max. unchanged ..	61	23	40	38	53	25
Ampl. max. decrease % ..	0	0	3	0	0	0

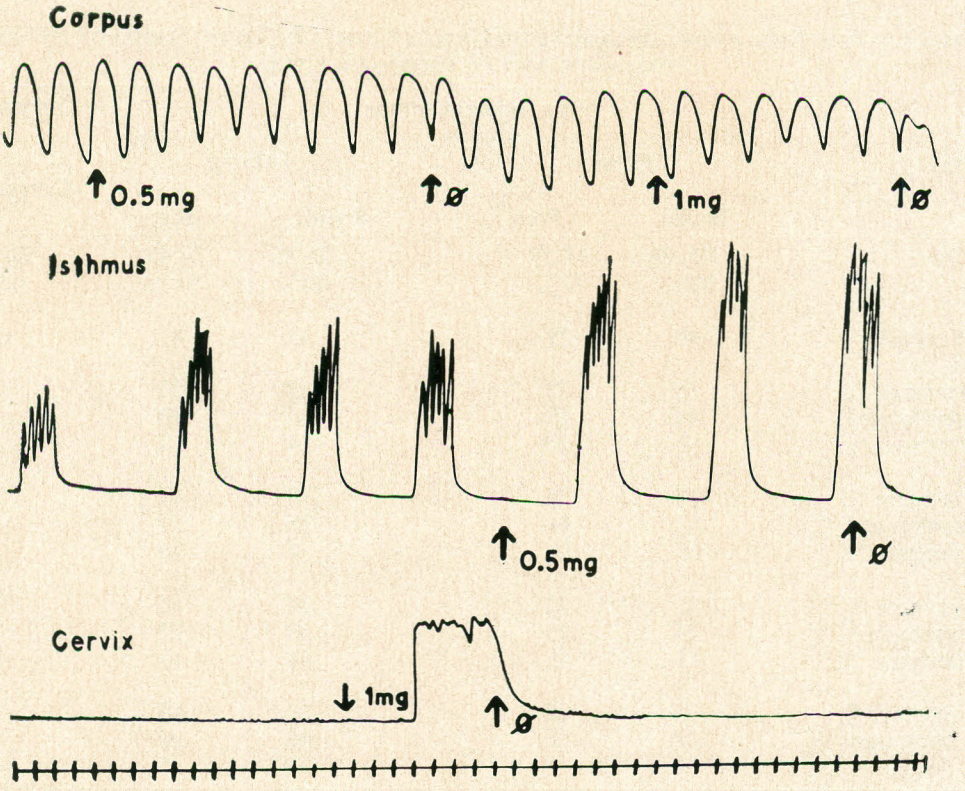


Fig. 5.—The effect of l-sparteine on the non-pregnant human uterus in vitro. The volume of the organ bath is 20 ml. φ=washing.

TABLE 3.—THE EFFECTS OF RETAMINE ON HUMAN UTERUS IN VITRO TESTED ON 32 NONPREGNANT AND 17 PREGNANT UTERI.

Parameter		Non-pregnant uterus				Pregnant uterus	
		Corpus		Isthmus			
		Prolif.	Secr.	Prolif.	Secr.	Corpus	Lower segment
Incidence of response		45/56	35/42	31/36	42/49	32/43	40/43
	..	80%	83%	86%	86%	74%	93%
Change of motility pattern %	..	22	20	32	38	3	43
To-nus	increase % ..	65	46	26	22	41	98
	unchanged %	22	43	58	52	53	2
	decrease % ..	13	11	16	26	6	0
Fre-quency	increase % ..	47	31	26	33	53	83
	unchanged % ..	40	40	19	30	31	15
	decrease ..	13	29	55	37	16	2
Ampl-itude	increase % ..	9	11	10	7	31	38
	unchanged % ..	40	52	26	24	44	38
	decrease % ..	51	37	64	69	25	24
Ampl. max.	increase ..	31	23	19	7	38	75
	unchanged % ..	47	60	23	17	59	25
	decrease % ..	22	17	58	76	3	0

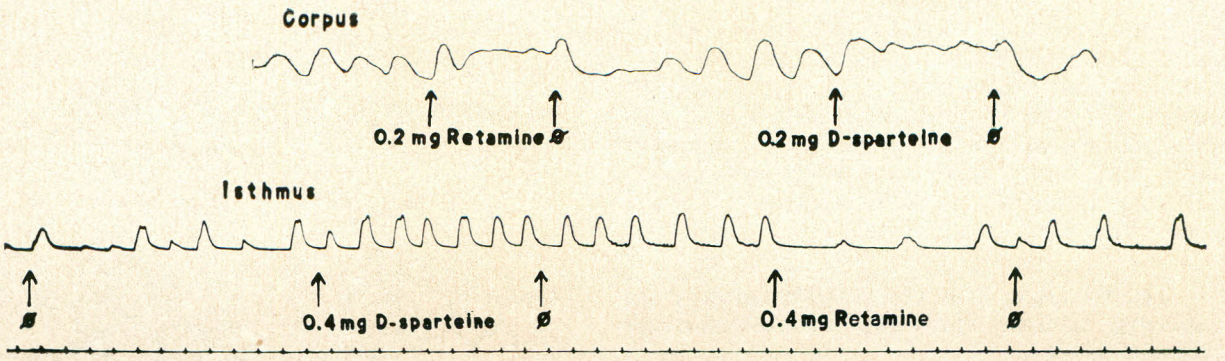


Fig. 6.—The effect of d-sparteine and retamine on the non-pregnant human uterus in vitro. The volume of the organ bath is 20 ml. φ=washing.

isthmus to various drugs has been found. These findings together with the observation of the qualitatively different response of the non-pregnant corpus and isthmus to retamine may support the assumption of a different structural or biochemical composition of the muscle cells in the corpus and the isthmus.

On the bas's of this experimental work investigations are now in progress to study the in vivo effect of retamine in woman to evaluate its possibility for the treatment of dysmenorrhœa.

The *d*- parteine has also been isolated from other plants of the Leguminosae family, among others *Sophora pachycarpa*, and has therefore also been given a synonymous name, pachycarpine. During the last 6-7 years this alkaloid has been used in Russia in the treatment of progressive muscular dystrophy (synonym: myopathy; myopathic atrophy).[9] The muscular dystrophies are a group of disorders of which the essential feature is a progressive degeneration of certain groups of muscles. *d*-Sparteine (pachycarpine), which is said to influence the autonomic ganglia, gives a good relief in the early stages of the disease.

Recently, Russian investigators have introduced another alkaloid in the combined treatment of myopathia and myasthenia gravis, namely galanthamine, belonging to the Amaryllidaceae alkaloid.[10]

Pancratium sickenbergeri Asch. and Schweinf. ex. Boiss, and Pancratium maritimum L.

We have investigated two species of the Pancratium genus, belonging to the Amaryllidaceae family.

The problems involved in the present investigation were the following.

1. To develop a suitable paper-chromatographic technique for separation of the Amaryllidaceae alkaloids in the plant material.

2. To compare the qualitative alkaloid content of the bulbs of *Pancratium sickenbergeri* and *Pancratium maritimum*.

3. To compare the qualitative alkaloid content of (*a*) the adventitious roots, (*b*) the bulb, (*c*) the vertical underground stem emerging from the bulb, and (*d*) the green leaves of *Pancratium maritimum*.

4. To compare the (qualitative) alkaloid content of the bulbs of *Pancratium maritimum* from various habitats (Egypt, Greece, France) during the same vegetation period.

Paper Chromatography—A method for separation of Amaryllidaceae alkaloids by paper chromatography has been described by Kincl, Troncoso and Rosenkranz.[11] This method was, however, unsuitable for our purpose, since the butanol-acetic acid system gives too small differences between the Rf values of the various alkaloids, whereas the benzene, chloroform and methanol systems produced more or less marked trailing in some cases.

A new method was therefore elaborated. The alkaloids as bases were separated by one-dimensional descending paper chromatography. The paper was impregnated with McIlvaine's standard buffer solution, pH 5.0, and secondary butanol saturated with buffer solution, pH 5.0, was used as solvent. The colour reagents used for detection of the alkaloids were iodine reagent and iodoplatinic acid reagent.

The chromatograms were run for 20 hours, and distinct spots were obtained.

The Dragendorff reagent could not be used for development of the chromatogram, since it reacts poorly with haemant idine, lycorine and tazettine under the present conditions. The use of two reagents affords a better possibility of identification, since they produce spots of different colour. The iodine reagent was useful especially for detection of the haemanthidine spot.

The R_f values and colour of the spots of the reference alkaloids and Pancratium alkaloids are listed in Table 4.

Alkaloids of Pancratium sickenbergeri and Pancratium maritimum.—Pancratium sickenbergeri has not earlier been investigated phytochemically. Bulbs of *Pancratium maritimum* collected in July on the Caucasian coast of the Black Sea were investigated by Proskurnina,[12] who isolated lycorine, tazettine and haemanthidine. From bulbs of *Pancratium maritimum* collected in December in Holland, Boit and Ehmke[13] isolated lycorine, tazettine and hippeastrine. The formulas of these alkaloids are given in Fig. 7.

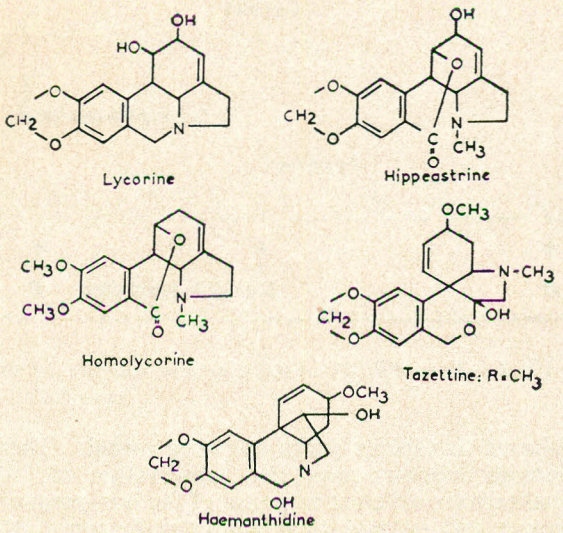


Fig. 7.—Alkaloids previously isolated from *Pancratium maritimum* L.

We have found that the bulbs of *Pancratium sickenbergeri* collected in April contain 8 alkaloids. In the bulbs of *Pancratium maritimum* of Egyptian origin, collected in May, the same 8 alkaloids were found, as well as one additional alkaloid. Of the 8 alkaloids in common, lycorine, homolycorine,

TABLE 4.—R_f VALUES AND COLOUR OF THE SPOTS OF REFERENCE ALKALOIDS AND PANCRATIUM ALKALOIDS.

Alkaloid	Symbol in Fig. 8	R_f value	Colour of spot	
			Iodine reagent	Iodoplatinic acid reagent
Reference alkaloids :				
Galanthamine	A	0.18	brown	dark violet
Lycoramine ..	B	0.19	,,	,, ,,
Lycorine ..	C	0.22	,,	violet
Lycorenine ..	D	0.29	,,	red-violet
Vittatine ..	E	0.31	,,	dark violet
Hippeastrine ..	F	0.37	,,	violet
Homolycorine ..	G	0.39	,,	blue
Haemanthidine ..	H	0.48	,,	red-brown, weak
Tazettine ..	I	0.60	,,	brown-violet
Pancratium alkaloids ..				
Alkaloid K ..	K	0.0	,,	blue
Alkaloid L ..	L	0.12	,,	dark violet
Alkaloid M ..	M	0.18	,,	,, ,,
Lycorine ..	C	0.22	,,	violet
Alkaloid N ..	N	0.30	,,	blue
Homolycorine ..	G	0.39	,,	,,
Alkaloid O ..	O	0.43	,,	dark violet
Haemanthidine ..	H	0.48	,,	red-brown, weak
Alkaloid P ..	P	0.53	,,	dark violet
Tazettine ..	I	0.60	,,	brown-violet

haemanthidine and tazettine were identified chromatographically and by physical data of the isolated alkaloids and some derivatives.

Until further identification, the other alkaloids are denoted as K, L, M, N, O and P.

Extraction.—All plant material, with the exception of the bulbs from Corsica, was extracted according to a modification of the method of Proskurnina.[12] Dried plant material 1000 g. was moistened with 1000 ml. of 5 M ammonia mixed with 500 g. of kieselguhr, and percolated with 75 kg. of ethylene dichloride ($ClCH_2CH_2Cl$). The percolate was evaporated to dryness in vacuo. The raw extract was extracted 3 times with a total of 250 ml. of warm 5 per cent H_2SO_4 The acidic solution was extracted with 2 times 100 ml. of chloroform and 2 times 100 ml. of ether. The solution was then made alkaline with concentrated ammonia. A precipitate appeared which was centrifugated, dissolved in 5 M acetic acid and reprecipitated with ammonia, and then washed with water (I). The mother liquors, together with the washing waters, were extracted with a total of 2000 ml. of chloroform. The chloroform extract was dried with Na_2SO_4 and evaporated to dryness in vacuo (II).

Fractions I and II were used for paper chromatographic separation of the alkaloids. Lycorine was obtained from I by recrystallization from methanol, and haemanthidine was obtained from II by extraction with hot acetone and recrystallization.

The yield of alkaloids from the different plant material, extracted according to the above procedure, is recorded in Table 5.

The bulbs of *P. maritimum* from Corsica were extracted according to the method of Boit.[14] 9.90 kg. of fresh bulbs were sliced, and extracted with stirring with 25 litres of ethanol (95 per cent) for 18 hours at room temperature. The alcohol solution was evaporated in vacuo to a syrup. The extraction procedure was repeated twice under the same conditions with 20 litres of ethanol. The total amount of syrupy residue was 1240 g. This residue was dissolved in hot water to a total volume of 2 litres. After acidification with H_2SO_4, the solution was extracted with 4 times 2 litres of ether and 4 times 2 litres of chloroform.

The aqueous solution was made alkaline by addition of concentrated ammonia. No precipitate appeared. The alkaline solution was extracted with 8 times 2 litres of chloroform. The emulsion formed separated by centrifugation. The chloroform extracts were dried with Na_2SO_4, filtered and evaporated in vacuo to a small volume, when lycorine precipitated, 1.99 g. The mother liquor was evaporated to dryness 13.9 g.

In our experience, Boit's method for extraction

TABLE 5.—ALKALOID CONTENT OF *Pancratium sickenbergeri* AND *Pancratium maritimum*.

Species	Part of plant	Weight		Alkaloid content			% lycorine
		Fresh g.	Dry g.	Fraction I g.	Fraction II g	Total % of dry wt.	calc. on dry wt.
P. sickenbergeri ..	bulbs	—	583	0.23	0.72	0.16	0.006
P. maritimum (Egypt)	roots	—	28	0.08	0.33	1.47	0.08
	bulbs	—	1370	4.57	4.44	0.62	0.11
	stems	—	299	0.91	0.95	0.62	0.11
	leaves	—	46	0.02	0.41	0.89	0.04
P. maritimum (Rhodes)	bulbs	1030	265	1.62	2.26	1.45	0.18
P. maritimum (Corsica)	bulbs	9900	(2500)*			0.64	0.08

*Calculated.

is superior to that of Proskurnina, since it is more rapid and economic, and also gives less impurities.

These two Egyptian *Pancratium* species, i.e. *Pancratium maritimum*, growing in the sandy dunes of the Mediterranean coast, and *P. sickenbergeri*, growing in sandy run-off furrows of the gravel desert in the Eastern part of Egypt, evidently have an almost identical qualitative alkaloid content.

Certain quantitative differences were, however, observed. Thus, the total alkaloid content of the bulbs of *P. sickenbergeri* was only one-fourth of that of the bulbs of *P. maritimum*. Furthermore, the bulbs of *P. sickengbergeri* had a low lycorine content and a high tazettine content. The reverse applied to the bulbs of *P. maritimum*.

Alkaloids in different parts of P. maritimum.—Previously, only the bulbs of different species of the Amaryllidaceae family have been studied phytochemically. However, we considered it of both theoretical and practical interest to compare the alkaloid content of the different parts of the plant. It was found that the adventitious roots, the bulb, the vertical underground stem emerging from the bulb and the green leaves of *Pancratium maritimum* had an identical qualitative alkaloid content, the afore-mentioned nine alkaloids being present in almost the same proportions (Fig. 8).

The only obvious quantitative difference was that the green leaves had a low lycorine content, and the adventitious roots had a high homolycorine content.

Alkaloids of P. maritimum from different habitats.—The influence of different habitats on the qualitative alkaloid content of *Pancratium maritimum* during the same vegetation period (May) has not been studied previously. We found that bulbs of this species collected on the Mediterranean coast (1) in Egypt, (2) on the island of Rhodes and (3) on the island of Corsica had 6 alkaloids in common (lycorine, haemantidine and the alkaloids L, M, N and O). Bulbs from Egypt contained, in addition, homolycorine, tazettine and the alkaloid K. Bulbs from Rhodes did not contain homolycorine or alkaloid K, and only traces of tazettine, but the alkaloid P, which was not present in the Egyptian and Corsican bulbs. Bulbs from Corsica contained no tazettine or alkaloid K.

The only alkaloid which has been identified in bulbs from all five habitats hitherto described (Black Sea, Holland, Egypt, Rhodes, Corsica) is lycorine. Obviously, this does not exclude the existence of other alkaloids common to bulbs from different habitats. A highly interesting finding is the absence of tazettine and alkaloid K in the bulbs from Corsica and of alkaloid K in the bulbs from Rhodes. The specific occurrence of alkaloid P and the high total alkaloid content of the bulbs from Rhodes is a noteworthy observation.

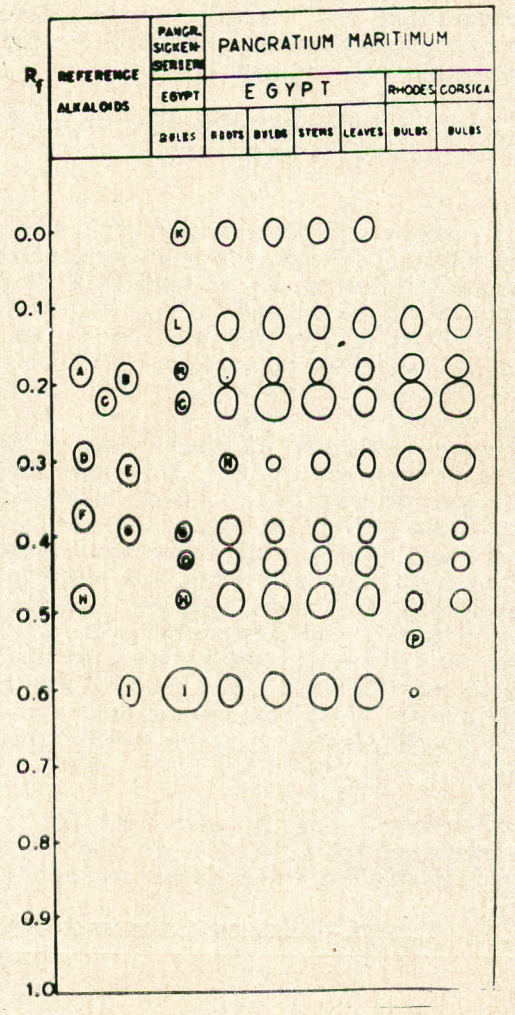


Fig. 8.—Alkaloids present in *Pancratium sickenberger* and *P. maritimum*.

The occurrence of the different alkaloids in the various plant material is illustrated in Fig. 8. The size of the spots shows in a semiquantitative way the relative amount of the alkaloids.

Investigations are in progress in our laboratory for further identification of the hitherto unidentified alkaloids occurring in the present plant material.

The pharmacological effects of the Amaryllidaceae alkaloids are, however, only poorly investigated. Lycorine, which is found in both Pancratium species investigated, is in fact the alkaloid with the most common occurrence in the family of *Amaryllidaccae.*

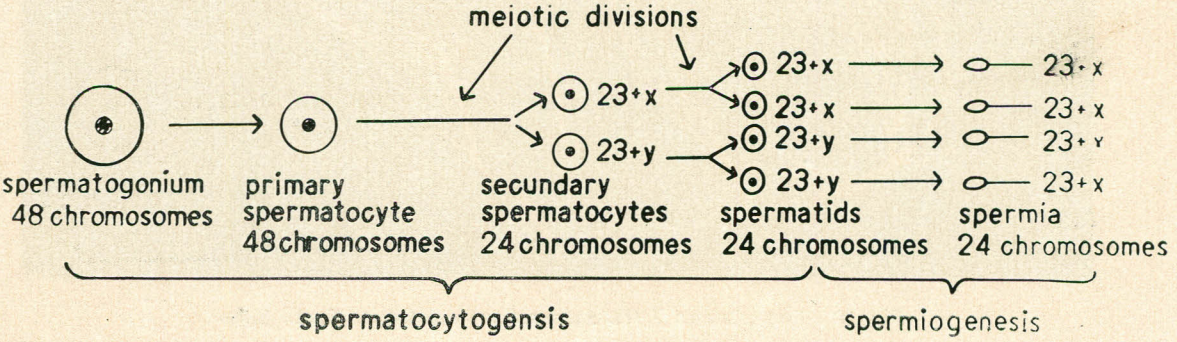
Some recent Japanese investigations are of special interest. Acute and chronic changes of blood picture caused by lycorine in rats were studied.[15] The leucocyte decrease was more marked than that of erythrocyte (which often was unchanged), and the minimum effective dose was approximately 0.02γ/100 g. bodyweight. The leucocyte decrease caused by lycorine was dependent on the neutrocyte decrease but independent of the lmyphocytes. (The most evident decrease in neutrocytes occurred in young cells rate of formation, while matured cells decreased markedly in absolute number). Histological investigation in the bone marrow in the middle part of femurs of treated rats showed the diminution in number and the cytological deformation of myeloblasts and little change in erythroblasts. Thus, there might be a potential clinical effect in the treatment of myeloid leukemia.

The effects of lycorine on the spermatogenesis of immature and mature rats has also been investigated.[16] Figure 9 shows the different stages of spermatogenesis.

In the immature rat after administration of 10γ/g. body weight for 10 days, it was found that lycorine most strongly affected the immature cells. While the development of the sperm cell in control animals had already reached the stage of the spermatid, the lycorine treated animals had stopped at the secondary spermatocyte stage. Cell division (mitosis) was seen only in the secondary spermatocytes, not in the spermatogonia and primary spermatocytes and no spermatid cells were observed. The normal cell division was interrupted in the secondary spermatocytes. Multinucleate cells were observed, these were of a giant form. The above nuclear changes were accompanied with those in the protoplasm, mainly in the form of vacuoles, with numerous minute juxtanuclear vacuoles appearing in the secondary spermatocytes.

In the mature rat, the testicular histological picture was not as marked as in the immature rats, with a slight decrease of cells in the seminal tubule, and a marked decrease in the immature cells i.e. spermatogonia and spermatocytes. The cell division was seen only in the secondary spermatocytes. The effect of lycorine in the testis of mature rats was different only in the degree of severity. These changes in testis caused by the continuous dosage of lycorine were not lasting after the adm nistration of lycorine was stopped. Thus, there might be a possibility to use lycorine as a contraceptive by making the man temporarily sterile.

The effect of lycorine seems mainly to be a blocking of some metabolic path way. This is supported by the fact that in the organs showing histological changes after lycorine (liver, adrenals, kidneys, testicles and ovaries) the ascorbic acid level is decreased. Thus, lycorine has the effect of decreasing the ascorbic acid levels in the main organs and causing scurvy, and their decrease differs with the individual organ. Since the administration of lycorine caused a decrease of ascorbic acid, then in rats given ascorbic acid these symptoms should not appear. In administering 1-100 γ/g. of ascorbic acid to rats given 5 γ/g. of lycorine, not only do the external symptoms such as loss of weight, haemorrhages and ulcers fail to appear, but the decrease of the neutrophils is not observed and the ascorbic acid levels of the main organs are normal; as an exception, the levels in the testes and ovaries do not return to normal. This may be because these organs expend more ascorbic acid than the other organs, or that lycorine does not only interrupt the ascorbic acid synthesis but acts on the immature cells damaging the cell itself and makes it impossible to reach the stage of ascorbic acid synthesis. If lycorine interferes with the ascorbic acid synthesis, then in humans and guinea pigs, which obtain the necessary ascorbic acid from the diet and have no synthetising mechanism, ascorbic acid deficiencies should not occur. On experimentally treating guinea pigs with lycorine, with the dosage and administration methods the same as in rats, no signs of decrease of body weight, haemorrhages, ulcers, and brittleness of the teeth which are seen in rats are observed and no decrease in the organ ascorbic acid levels is seen. This is the



The different stages of spermatogenesis

Fig. 9

exact opposite to rats and this substantiates the fact that the action of lycorine is on the ascorbic acid biosynthesis.[17]

Haloxylon salicornicum (Moq.-Tand.) Boiss.

In our continued phytochemical studies of the Egyptian flora, we have investigated *Haloxylon salicornicum* (Moq.-Tand.) Boiss.[=Haloxylon schweinfurthii Boiss., Hammada salicornica (Moq.-Tand.) Iljin], belonging to the Chenopodiaceae family.

The genus Haloxylon comprises 13 species growing in the arid zone of the North-African and Arabian Deserts and in South-West Asia. *H. salicornicum*, which is a typical desert plant of xerophytic nature (Fig. 10) with the aforementioned distribution, has not earlier been investigated phytochemically. According to Engler and Prantl,[18] this species is used by the natives of South-West Asia as a remedy for diseases of the respiratory tract, especially coryza. The vapour fom the burning plants is inhaled. The plant was mentioned by Ibn el Baithar (d. 1248) under the name of "Rimth."

The only species of the genus Haloxylon that has previously been studied phytochemically is *H. tamariscifolium* Pau *Haloxylon articulatum* (Boiss.) Bunge]. In his thesis, Rosengart-Famel[19] showed this species to contain two alkaloids; one with the formula $C_{11}H_{16}O_2N$, m.p. 176°C., and the other—which was liquid—with the formula $C_{10}H_{18}O_2N$. No further identification has been made. These alkaloids are most probably of another type than those present in *H. salicornicum*.

Since we found the alkaloid content of this plant to have a somewhat complex pattern, we have considered a preliminary report to be justified. Further data on the different alkaloids will be given in a later paper.

The plant material was mixed with ammonia and extracted with a chloroform-ether mixture. After evaporation of the solvent, the alkaloids were extracted with dilute hydrochloric acid. The acidic aqueous solution was made alkaline with sodium hydroxide. The alkaline aqueous solution was thoroughly extracted with ether to obtain the ether-soluble, most probably tertiary, alkaloids. After this procedure, the solution still contained alkaloids which could not be extracted with ether, presumably phenolic or quaternary alkaloids.

In order to ascertain the number and relative amounts of ether-soluble alkaloids, they were

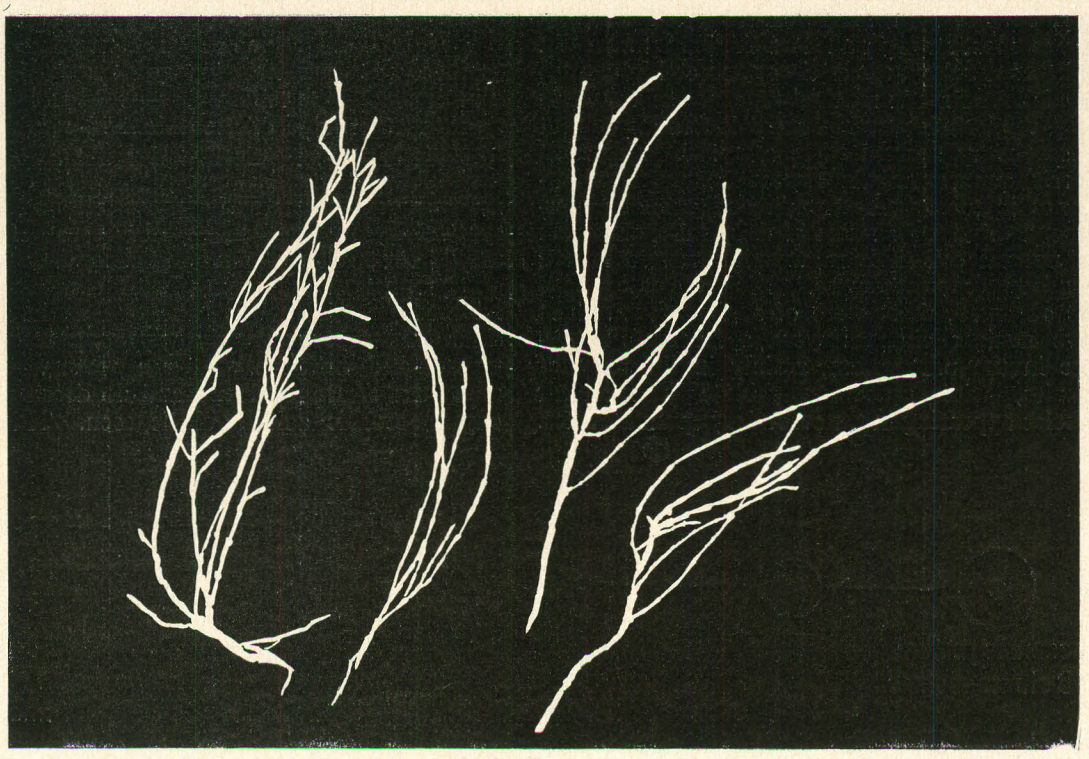


Fig. 10.—Part of stems of *Haloxylon salicornicum* (Moq. Tand.) Boiss.

separated preparatively on a cellulose column with a solvent mixture of *n*-butanol-formic acid-water. The fractions collected were checked by paper chromatography with the same solvent (Fig. 11). This showed the presence of eight alkaloids.

Since the alkaloids obtained from the cellulose column were difficult to crystallize, the alkaloid mixture was separated on an alumina column with ether, followed by ether with 1% and 5% methanol. The main alkaloid with the highest Rf value was obtained pure from the ether solution, and was isolated as hydrochloride: $C_{16}H_{26}N_2O_2.HCl$, mp. 193.5-196°C.

After the extraction of the ether-soluble alkaloids, the afore-mentioned alkaline aqueous solution was made acidic. The phenolic or quaternary alkaloids present were precipitated with reineckate, transformed to chlorides and separated on a cellulose column with a solvent mixture of *sec.* butanol-formic acid-water. The fractions collected were checked by paper chromatography with the same solvent. This showed the presence of eight alkaloids.

The mixture of the ether-soluble alkaloids is fairly toxic, the LD_{50} in mice after intraperitoneal administration was 6.2 mg./kg. The absence of pronounced autonomic effects was a characteristic feature of these alkaloids. The blood pressure is almost unchanged at moderate doses and it gives a peripheral vasodilatation, tested on isolated rabbit ear, and in doses of 1-2 mg./kg. it decreases the spontaneous motility of rats, indicating a sedative effect.

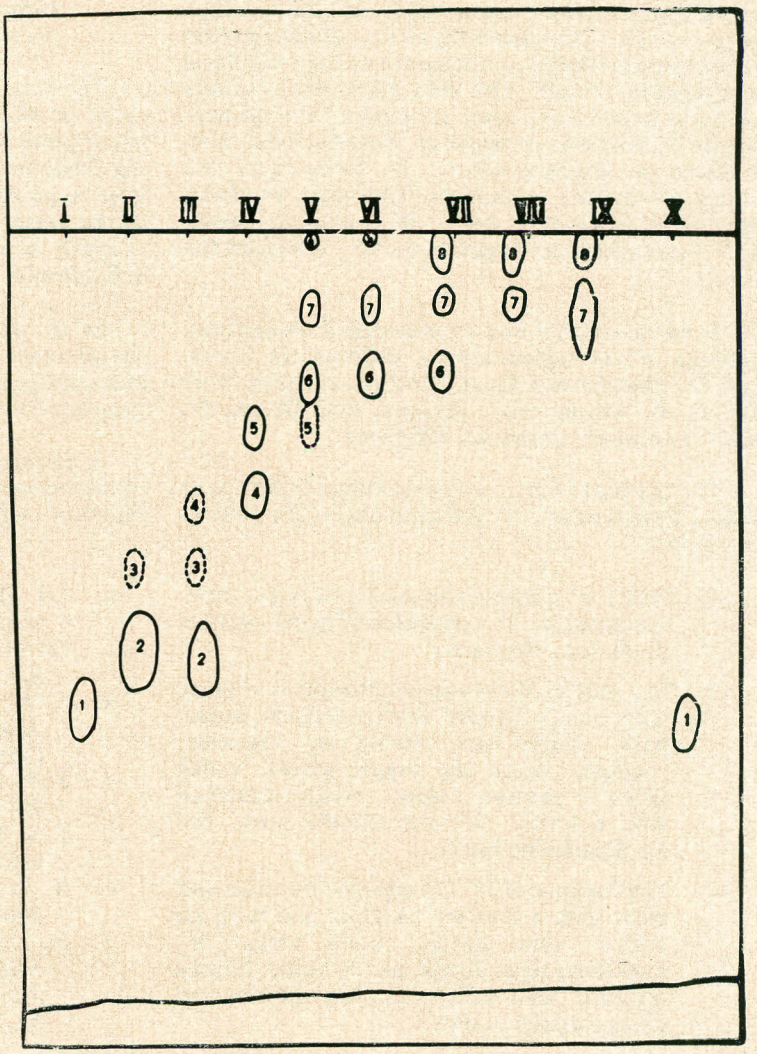


Fig. 11.—Paper chromatography of fractions I-IX of the ether-soluble alkaloids separated on a cellulose column. X is the main alkaloid obtained from an alumina column. For solvent, see text.

Withania somnifera (L) Dunal

This species grows wild in waste places of the Nile-Valley and Delta region and Sinai. The root of this plant is used by the Egyptian public as a remedy for rheumatism, the leaves as a fibrifuge and the fruits as a diuretic. In East Africa it is regarded as a narcotic and antiepileptic. It has been investigated by Fahmy,[20] and F. B. Power and A. H. Salway,[21] but they were unable to detect any of the solanaceous alkaloids.

From Indian cultivated plant material, Majumdar[22] has isolated eight alkaloids: nicotine, somniferine, somniferinine, somnine, withanine, withananine and pseudowithanine. However, according to Kaul,[23] the cultivated plant investigated by Majumdar, differs from the wild one not only in its therapeutical properties but in all its morphological characters like roots, stems, leaves, flowers, pollen grains, mature fruits, seeds and the enlarged calyx to such a great extent that the cultivated plant should be considered as a separate species, *Withania ashvagandha* Kaul. The wild plant *W. somnifera* Dunal is commonly found growing as a weed near human habitations throughout Western India from Kashmir to Western Ghats. In India these two plants are known to modern Ayurvedic practitioners as Ashvagandha desi(= *W. somnifera* Dunal) and Ashvagandha nagouri (=W. ashvagandha Kaul).

A macroscopical and microscopical comparison between *W. somnifera* Dunal, collected in Egypt, and *W. ashvagandha* Kaul, obtained in India from Dr. D. N. Maujumdar, Benares, and Dr. K. N. Kaul, Lucknow, shows the following.

The tap root of *W. somnifera* Dunal from Egypt differs from that of *W. ashvagandha* in the following points :

1. While *W. ashvagandha* tap root is 2-12 mm. in diameter, *W. somnifera* (Egypt) reaches in 90 mm. diameter.
2. The root of *W. somnifera* (Egypt) is covered with comparatively thick, reddish brown bark which can easily be removed, specially from the bigger pieces, which gives a pinkish colour when scratched with a knife. *W. ashvagandha* root has no detachable bark.
3. The fracture of *W. ashvagandha* is short and clear with a starchy interior and with no odour and starchy acrid taste. *W. somnifera* from Egypt has a hard, fibrous fracture with yellowish white interior and characteristic odour.
4. The starch of both roots is more or less similar but the compound starch grain in ashvagandha is more than 60%. In the *W. somnifera* from Egypt, the simple ones are more than 60%.
5. In the mature main roots of *W. ashvagandha*, parenchymatisation of the xylem takes place starting in small patches in the central region of the root, and it gradually increases outwards, and finally all the xylem elements except the vessels as well as a few tracheids become non-lignified and parenchymatous. The immature and the daughter roots do not undergo this interesting change and possess a normal central solid, thickened and lignified wood core. In *W. somnifera* (Egypt), the main mature as well as the daughter roots, the xylem shows the ordinary normal and complete lignified and thickened wood core.

The leaves of *W. ashvagandha* are ovate while those of *W. somnifera* (Egypt) are broadly ovate, but a great similarity exists, and the most interesting difference which can differentiate between them even in powder condition is that the anticlinal walls of the upper and lower epidermises of *W. somnifera* (Egypt) is distinctly beaded while that of *W. ashvagandha* is not.

In the root of Egyptian *W. somnifera* Dunal, the presence of five alkaloids has been showed by chromatographic technique. Investigations are in progress for the identification of these alkaloids.

A mixture of all alkaloids in doses of 5 mg./kg., intraperitoneally on rats, showed a pronounced inhibitory effect on the spontaneous activity.

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