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Chemical Pharmacology of *Catha Edulis*

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Catha edulis, the Arabic khat, the Amharic or Ethiopic tschat, or the English qat, was probably grown and used as a food stimulant in the present area of the Ethiopian highlands around Harar from the earliest times. It grows as a bush or tree, the stem tips and leaves of which are consumed today in certain regions of East Africa, in Aden, and in Yemen. Its use is confined to those areas in which it is grown or to which it can be transported in the freshly cut condition. Its principle use is as fresh plant material, the more succulent parts of which are chewed and then swallowed. Some limited use is made of the dried powdered leaves steeped with water as a tea, or eaten as a paste with honey or other sugary binder.[1]

The first historical reference to khat appears in a chronicle on Amda Seyon, who reigned in Abyssinia from 1314–1344.[2] This relates that Sabr Ad-din, then Arab king of Ifat, before embarking on a war with Amda Seyon, said, 'As for Marade, his capital, I shall make it mine and shall plant khat there because the Muslims like it.' According to d'Hericourt[3] the planting of khat was introduced from Abyssinia into Yemen about 1424 by Sheik Abou Zerbin. Reference to its use then being extended from Yemen to Aden is found in the writings of the Arab Abdul-Kadir.[4] Its cultivation and use in Ethiopian and South-western Arabian areas is considered to be earlier than that of coffee.[5]

The khat plant was first classified by Forskål, the botanist who started with the Niebuhr mission into Yemen in 1762. He described the plant under the name of *Catha edulis* and classified it along with an additional species then designated as *spinosa*.[6] Forskål noted its cultivation along with *Coffea* and reported that the Arabs in Yemen ate the green leaves and ascribed medicinal virtues to the plant material. Present-day classification[7] places the genus *Catha* in the family Celastraceae and recognizes but one species, *Catha edulis*, Forsk. The species name *edulis* was repeated in a separate but now obsolete classification of the plant as *Celastrus edulis*

by Vahl in 1790.[8] Quartin-Dillon[9] designated the plant material as Thé des Abyssins or Abyssinian Tea and it has also been called African or Arabian Tea.

The attention of the English world was directed to the stimulating effects and Arabian usages of khat by Vaughan in 1852.[10] He was then port surgeon at Aden and in his drug notes remarked on the strong interest that Arabs had for this material. It was then brought down from the mountain areas north of Aden on camel back almost daily. Then, as now, the English government taxed the selling of it in Aden.

Vaughan noted that, as with coffee, the use of khat had been the theme of Moslem reasonings, and names of Arabian renown have reasoned as to whether the use of khat does, or does not, oppose the injunction of the Koran that 'thou shall not drink wine or anything intoxicating'. One godly and learned Ali Shadeli ibn Omar introduced coffee into Aden at a time when khat was not available because of a drought, as producing the same effect with less expense and trouble. Khat as well as coffee has continued as a theologically accepted and lawful custom in adjacent areas of Arabia and Africa to the present day. Air transport has made Harar in Ethiopia the agricultural centre of supply, other than locally in Yemen and in some parts of East and South Africa.[1,11,12]

Elucidation of the chemical nature of the pharmacologically active substances present in Catha materials has been periodically attempted. Flückiger and Gerock in 1887[13] repeated some earlier works [14-17] and found caffeine is not present. However, they did find the presence of a small amount of a more basic material which they termed 'katine'.

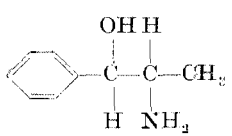
Mosso in 1891[18] separated a basic fraction as a hydrochloride in solution but was not able to crystallize this or any other salt. He called his base fraction 'celastrina' and found its solution to exert a stimulant action on isolated frog heart. Injection of a minimum lethal dose into a frog caused dilation of the pupils, some increased motor and respiratory activities, then loss of coordination of movements and tremors of the extremities. Convulsions, then rigidity of extremities, and finally respiratory arrest resulted. The pharmacological comparison by Mosso[18] of these several effects with those of cocaine has been variously quoted later. Such quotations out of proper context have contributed to present-day illogical assertions of the presence of 'narcotic' actions among khat materials.

Beiter in 1901[19] presented chemical evidence that extractable basic materials are indeed present. He also noted the considerable quantities of tannins present that he characterized as being

like those present in Chinese and Indian teas. The basic material isolated had the characteristics of a strong base and he was able to obtain some hydrochloride, hydrobromide and salicylate crystalline materials, however in quantities insufficient for complete chemical analyses. Ten and twenty milligram injections of the base materials were observed to cause a paralysis in frogs. The yield of bases isolated was only 0·034 per cent in leaves from Harar and 0·076 per cent in leaves from Aden.

The chemical pharmacology of Catha was notably confused by the inept chemical work and reporting of the pharmacologist Stockman in 1912.[20, 21] He contrived to separate three fractions from making the extracts basic—two of these finally in the form of crude crystalline sulphate materials. The third was a fraction insoluble in water when obtained by making alkaline a water extract of the plant. These various preparative fractions were not adequately defined either by details given of their isolation or by the analyses or properties of product obtained. Nevertheless, Stockman gratuitously gave individual names to the crude fractions and proceeded to report some effects observed with these named materials in animals.[21] His work has been variously and uncritically quoted since that time, and his naming of products repeated as if they were definitive of chemical substances.

It remained for Wolfes in 1930[22] to isolate and identify a pure base from Catha plant material. This followed the advent of ephedrine into Western medicine and Wolfes found *dextro*-norpseudoephedrine, (+)-*threo*-2-amino-1-phenylpropanol (I), as an extractable base material in Catha.



(I)

N-Methylation of this compound yields *dextro*-pseudoephedrine, a stereoisomer of naturally-occurring *levo*-ephedrine. *Dextro*-norpseudoephedrine as well as *dextro*-pseudoephedrine have been found present in some species of Ephedra.[23–25]

Though Wolfes established that *dextro*-nor-pseudoephedrine was present in the unspecified Catha material he used, its possible

amount and potential pharmacological activity were neither reported nor commented upon. Whether this substance might, or might not, account for the use of Catha plant materials was not developed from the work done. Opinions based on inadequate data have since been expressed on these questions. Von Brücke[26, 27] considered that if all the base content established by Beiter[19] were the *dextro*-nor-pseudoephedrine, its amount would be quite inadequate to explain its stimulant activities. On the other hand, Hoffman and co-workers[28] felt from their work on the effects of synthetic nor-pseudoephedrine isomers in man that the presence of *dextro*-nor-pseudoephedrine in Catha materials might explain its usages. A recent review article[29] places emphasis on the possibility of as yet unidentified chemical constituents being responsible.

Catha Materials Used

A selection of some available fresh grades of khat was made by Alles at the Dire Dawa market in Harar province in Ethiopia during December of 1955. A representative selection was made on the advice of local residents engaged in marketing or shipping and taxing of export materials from that area. The main lot selected was of the grade called Quaranga which is most commonly consumed in Harar because of its lower local price. Some of the more costly Kuda and Karthe grades which are of a younger and more succulent growth were also obtained. These two grades are more commonly used for the highly taxed air-transport from Ethiopia and again taxed import into French Djibouti and British Aden.

Quantities of these materials were separately dried in the equatorial sun and air for two days. The dried materials were put into canvas bags and sent by plane in transit through Aden, then by ship to London, and finally to Los Angeles. The received products were finely powdered in a hammer mill before extraction.

In the spring of 1956, some ends of stems with leaves were cut from imported Catha trees that had been growing for a long time south of Miami in Florida. This material was obtained with the generous help of Dr. Margaret J. Mustard of the University of Miami Department of Botany and of Dr. R. Bruce Ledin of the University of Florida Subtropical Experiment Station.

Some cuttings from the Florida-grown plants were rooted in Pasadena, California, and stems with leaves from these plants were also obtained for analyses in the winter of 1957. A large well-growing bush of Catha was later located in the Botanical Garden of the University of California, at Los Angeles. Air and sun drying of this fresh material caused about a two-thirds loss of weight, or 68 per cent on the average.

Estimations of Total Extractable Bases

Some relatively high values for total bases were obtained from estimations based on the use of continuous benzene extraction of alkalinized aqueous extracts.[30] This method is quite effective but is only accurate for estimation of ephedrine and like bases in simple preparations. When applied to plant materials such evaluations substantially include some of the considerable amounts of ammonia that may be in such materials and were found to be present in Catha materials.

Dextro-nor-pseudoephedrine, along with some other ephedrine and norephedrine isomers, is present in some Ephedra species[23, 24, 25] and contributes to the total extractable bases of such plants. Therefore, the official method for estimation of total bases in Ephedra, adopted by the Association of Official Agricultural Chemists,[31] was applied to our Catha materials. These estimations do not include more than minimal amounts of the ammonia also present, due to its almost complete removal by evaporation of the extraction solvent that is employed, before the addition of any acid. The following results were found with the Catha materials studied:

Air-dried sample	Total bases,[a] %
Khat, Quaranga, main lot	0·11
Khat, Kuda grade	0·14
Khat, Karthe grade	0·16
Catha from Florida	0·02
Catha from California	0·02

[a] Calculated as a norephedrine base.

These values may be compared with the 0·17 and 0·18 per cent of the extractable bases estimated on hot chloroform extractions of some air-dried Ethiopian and Tanganyikan plant samples by Paris and Moyse.[32] Their later studies of materials from the Antibes and Beirut gave 0·18 and 0·20 per cent of extractable bases.[33] All of these amounts were presumably calculated as ephedrine base and possibly included some ammonia.

Preliminary estimations of extractable and volatile bases were carried out on extracts made by refluxing a 20 g sample with 200 ml of water for

10 min, then taking a 25-ml aliquot of the filtrate. After making alkaline with 5 ml of 2·5 N NaOH the bases present were extracted with benzene in a continuous extractor.[29] The extracted solution was directly titrated with 0·10 N perchloric acid in dioxane, using methyl red indicator. The total base found present was 0·48 mmoles for a 20 g sample of the main lot and represented ammonia plus any ephedrine type bases. Steam distillation of a similar aliquot amount led to an estimation of 2·14 mmoles of volatile base for a 20 g sample and consisted principally of ammonia, though a trace of nor-pseudoephedrine is volatile with steam. With the Kuda and Karthe grades, higher estimations resulted for both extractable and volatile bases while much lower values were obtained for the Florida-grown material.

Following the A.O.A.C. method[31] for estimation of total extractable bases in Ephedra plant materials, with the chloroform–ether extractable applied directly to alkalinized plant samples, consistent estimations can be obtained that include only negligible amounts of ammonia. Estimated base totals about 0·16 mmoles for a 20 g sample of the best grade material and the method systematically applied to Catha samples gave the results reported in the table.

The titration solutions resulting from the estimations were each evaporated so that 1 ml was from 5 g of plant material. Samples of 2 μl each on chromatography paper were distributed by descending flow of solvent for about 4 h. A freshly prepared 60 : 25 : 15 butanol–acetic acid–water solvent was used. On adjacent areas 3 × 2 μl samples of 0·01 N nor-pseudoephedrine and pseudoephedrine salts in solution were distributed. Testing with ninhydrin reagent showed similarly R_f-valued spots with each of the Ethiopian plant samples and with the nor-pseudoephedrine and pseudoephedrine.

Testing of similarly distributed areas with the Baker[34] acetoacetylphenol reagent and ultraviolet examinations showed the same R_f spots except with pseudoephedrine. This accords with the specificity of this reagent for primary amines and evidences a primary amine at this R_f for these samples.

The R_f values for these bases depend on the amount of material placed on the paper. With 2 μl of 0·01 N solution the nor-pseudoephedrine value is about 0·73–0·74 while with 2 × 2 μl of 0·10 N the larger areas centre about R_f 0·68.

When 10 × 2 μl amounts of the 5 : 1 titration solutions were compared with 3 × 2 μl amounts of 0·10 N nor-pseudoephedrine similarly R_f-valued spots with comparable intensity were noted after the ninhydrin reaction.

When 6 × 2 μl amounts of the Florida and California titration samples were used for distribution, the presence of the same base was evidenced, but in much lesser amounts. With 6 × 2 μl amounts of any of the Catha samples studied no test spots were evidenced with Dragendorf, Wagner or diazotized sulphanilic acid reagents. Absence of reactions with the latter indicates that no considerable amounts of imidazole, indole or phenolic bases are present in the Catha materials.

Estimations of Tannins

Beiter[19] noted the marked astringent and bitter qualities of aqueous extracts of Catha materials and ascribed these qualities to the presence of notable amounts of tannins that gave blue and green colourations with ferric iron salts. These tannin materials could be precipitated by suitable additions of lead salts. The similarities between these tannins and those that occur in Thea species were remarked upon by Beiter. Recently, Paris and Moyse[32] estimated that their Catha sample from Tanganyika contained about 14 per cent tannins, based on precipitation with the Stiasny reagent composed of formaldehyde and hydrochloric acid.

Short-time steeping of our Catha samples with hot water using amounts of 5 to 15 g/l. gave partial extracts that were acceptably swallowed as a tea. Extracts made by boiling even 1 or 2 g/l. mixtures for some time are so astringent and bitter that they could not be taken in quantity. The astringent and bitter qualities are largely due to the content of tannins in the Catha materials, but a bitter-sweet and rather unpalatable quality remains notable in concentrated solution after lead insoluble tannins have been removed. Concentrated extracts of Thea materials exhibit similar strongly astringent and bitter qualities.

The A.O.A.C. official method for the estimation of tannins in Thea materials[35] is based on the Proctor gelatin precipitation reaction and this method applied to our powdered Catha materials gave the following results:

Air-dried sample	Total tannins,[a] %
Khat, Quaranga, main lot	7·4
Khat, Kuda grade	5·5
Khat, Karthe grade	7·9
Catha from Florida	5·6
Catha from California	6·0

[a] Calculated as a gallotannic acid.

Isolation of *Dextro*-nor-pseudoephedrine

Definitive advance in our chemical knowledge of the nature of a pharmacologically active material in khat or Catha materials was made by Wolfes in 1930.[22] He isolated some *dextro*-nor-pseudoephedrine and identified it in the form of the base and its

hydrochloride. However, he offered no estimate of the amount present in, or isolatable from, his inadequately identified plant material. Our first attempts to follow the minimal description of his isolation method, using hot benzene extraction of limed material, did not yield enough basic material to isolate and identify.

By extending the A.O.A.C. total base estimation method to a larger scale operation, a satisfactory isolation procedure resulted with the isolation of about 0·1 per cent of *dextro*-nor-pseudoephedrine in the form of its optically pure sulphate. Its salt with dibenzofuran-2-sulphonic acid is useful for identification comparisons with related nor-ephedrine isomers and also with the isomers of ephedrine.

The 200 g main lot of powdered khat was shaken with a mixture of 500 ml of chloroform and 1500 ml of ethyl ether for a few minutes and then alkalinized with a mixture of 50 ml of 20 per cent Na_2CO_3 and 50 ml of 15 N NH_4OH solution. After 4 h of occasional shaking the mixture was filtered and the residue washed with two 200-ml portions of ether.

The solvent filtrates were combined and shaken out with two 100-ml portions of 0·50 N H_2SO_4. The aqueous acid extracts were combined and 200 g of powdered Na_2CO_3 was added, then the mixture was shaken out with five 100-ml portions of ether. The ether extracts were filtered through dry paper and then evaporated in a stream of room air. An aqueous condensate resulted and this required 3·0 ml of 0·50 N H_2SO_4 to adjust to pH 4·8. This titration represents 0·11 per cent of base, calculated as a nor-pseudoephedrine, in the Catha material used.

The aqueous product from five 200-g batches was treated with some decolourizing carbon, filtered, then evaporated to a 20 ml volume. Addition of 200 ml of acetone precipitated a crystalline solid almost white in colour. This was taken up in 10 ml of hot water and 90 ml of ethanol, then 150 ml of ether was added. A flaky white crystalline sulphate weighing 1·10 g resulted, m.p. about 292° (d.), $[\alpha]_D^{25} + 90·0°$ ($c = 2\%$, in water).

Comparison with a synthetic sample of *dextro*-nor-pseudoephedrine sulphate, derived from resolution of racemic base with D-tartaric acid followed by a conversion to sulphate, showed this to melt at about 293° (d.) With 0·200 g made up to 10·0 ml in water the rotation was +0·80° at 25°, or a specific rotation of $[\alpha]_D + 40·0°$ under the observed conditions. The 0·11 per cent of sulphate that had been isolated from the Catha material used represents an isolated yield of 0·083 per cent. of *d*-nor-pseudoephedrine base. The physical constants of the synthetic and isolated sulphates compare well with those reported by Nagai and Kanao[24] who gave m.p. 290–291° and $[\alpha]_D^{27} + 39·66°$.

As with the optically isomeric ephedrines,[36] the dibenzofuran-2-sulphonic

acid salts of the norephedrines and nor-pseudoephedrines were found to crystallize well from water and to exhibit usefully characteristic melting points. The 10 ml solutions from optical rotation determinations on the sulphates were each poured into hot solutions of 5 ml of 0·001 M dibenzofuran-2-sulphonic acid. After cooling, the crystalline salts separated and were filtered off. The isolated *d*-nor-pseudoephedrine salt melted at 221–222° and the synthetic salt at 222–223°. A mixed sample melted at 221–222°.

Dibenzofuran-2-sulphonate from	m.p., °C
l-Ephedrine HCl of $[\alpha]_D - 35 \cdot 0$ at 23°	205–206
d-Pseudoephedrine HCl of $[\alpha]_D + 62 \cdot 0$ at 22°	280–281
l-Norephedrine HCl of $[\alpha]_D - 34 \cdot 0$ at 24°	245–246
d-Nor-pseudoephedrine HCl of $[\alpha]_D + 42 \cdot 8$ at 26°	221–221

Chromatography on paper of 1 and 2 μl amounts to 0·1 N solutions of the isolated and synthetic nor-pseudoephedrine sulphate samples showed identical R_f values for the single spots after solvent distribution. They were made visible with ninhydrin or the acetoacetylphenol reagent having specificity for primary amines.[34]

When larger amounts, 1 and 2 μl of 1·0 N solutions which contain 0·15 and 0·3 mg of norephedrine or ephedrine bases, are applied to the paper, two separately developed spots of clearly different R_f values and shade with ninhydrin reagent result with each single compound. Paris and Moyse[32] noted this appearance of two different R_f values with their crude base extracts from Catha and erroneously interpreted this to mean the presence of two different bases, Base No. 1 and Base No. 2, in the plant material. In comparison, they only used a 2 per cent solution of ephedrine hydrochloride, which does not clearly show the two R_f values for the single base substance, except with the application of large volumes. The two-spot phenomenon is shown with similar large amounts of most bases applied to the paper and appears to represent some difference in solvent transfer of the base ion and of an undissociated salt.

The combined filtrates from the isolation of the crystal sulphate from acetone and from the recrystallization of the material from ethanol–ether were evaporated to dryness and then taken up with 5 ml of water. A pale yellow solution resulted and some yellow-brown resinous material which remained undissolved was filtered off. Chromatography of the filtered 200 : 1 aqueous solution showed only some ninhydrin and acetoacetylphenol positive material of R_f value comparable to nor-pseudoephedrine. Some yellow spot material was noted at R_f values of 0·92–0·96, as reported by Paris and Moyse,[32] gratuitously as Base No. 3. This material shows neither ninhydrin, acetoacetylphenol or Dragendorf reagent reactions. This area may also show yellow-green colours that are apparently chlorophyll-derived materials.

Absence of Amphetamine

A few mice were injected by von Brücke[26, 27] with some *dextro*-nor-pseudoephedrine sulphate. Only a small quantity was available to him and with single mice he reported that 50 mg/kg subcutaneously did not cause any obvious motor activity while 100 mg/kg did. Under similar conditions von Brücke reported that 0·50 mg/kg of *dextro*-methamphetamine hydrochloride usually causes an increase in motor activity and that 1·0 mg/kg always did. Based on the estimations of Beiter[19] that Catha materials contain only 0·034 to 0·074 per cent total bases, von Brücke concluded that the presence of *dextro*-nor-pseudoephedrine would not be adequate to explain the stimulant activity of the plant in man. When he took 10 mg of the sulphate by mouth, followed in an hour by 20 mg subcutaneously, he observed no stimulant effect greater than that which may follow a cup of coffee.

The close structural relationship of the amphetamines to the nor-pseudoephedrines led von Brücke[26, 27] to suggest that an amphetamine might possibly be present in effective amounts in Catha materials. This suggestion would now appear even more plausible since Leete[37] has been able to show with radioactively tagged phenylalanine that this amino acid is converted into *dextro*-nor-pseudoephedrine in the biogenetic metabolism of *Catha edulis*. Should the total reduction of the carboxyl group of naturally-occurring phenylalanine be the first step in such a conversion, *dextro*amphetamine would be present in the plant as an intermediate compound.

Racemic or *dextro*amphetamines, or the isomeric nor-pseudoephedrines, do not greatly differ in the R_f values of their chromatograms with the usual solvents. When freshly prepared 60 : 25 : 15 butanol–acetic acid–water solvent is used on 2 μl amounts of 0·01 N solutions of these bases the R_f values are about 0·74 and 0·79, respectively. The ninhydrin test responses of the spots from amphetamines are much less intense than from the same amounts of norephedrines. Aqueous solutions of the total extractable bases of Catha materials in dilute solutions of about 0·01 to 0·1 N, readily show a single spot centring about R_f 0·72–0·73 and any small amounts of material centring about a slightly greater R_f cannot be distinguished. When larger amounts, four

or more μl of 0·1 N solution or a few μl of 1·0 N solution are put on the paper, the presence of two spots in this range of R_f values is noted even with pure *dextro*-nor-pseudoephedrine alone. Under such conditions it is again not possible to distinguish whether or not such substances with nearby R_f centres are present in small amounts.

Further difficulties are present when chloroform–ether or ether extractions are used to obtain the total bases from the plant materials. When such extracts are partially or wholly evaporated to remove ammonia before acidification, amphetamine bases could be lost to a considerable extent if they were present. It was necessary to develop a definitive method without using solvent extractions and their evaporation.

A steam distillation method was developed that strongly favours the separation of any amphetamine from the comparatively large amounts of the nor-pseudoephedrine present. This was followed by chromatographic comparisons of the acidified distillate after suitable concentration. With such a method, the base of as little as 0·10 mg of amphetamine sulphate added to 10 g of powdered plant sample was detectable by a margin of more than twice in the spot amounts tested. The plant material alone yielded no evidence of amphetamine. Amphetamine was therefore absent, or rather no more than half of 0·01 per cent of amphetamine sulphate equivalent can be present. In other words, no more than 5 mg of racemic or *dextro*amphetamine sulphate equivalent could be present in 1 kg of dry or 3 kg of fresh plant material.

A 10 g main lot of powdered khat was mixed with 10 ml of 20 per cent Na_2CO_3 solution and 190 ml of water, then distilled from a flask into a receiver containing 1·0 ml of 0·40 N H_2SO_4 and 2 drops of methyl red indicator solution. When the indicator showed neutrality, the distillate was removed and evaporated to dryness, then taken up with 0·4 ml of water to yield a neutralized 1·0 N solution of volatile bases. This solution was chromatographed on paper using the 60 : 25 : 15 butanol–acetic acid–water solvent. The paper was equilibrated above the solvent for 16 h and then developed with descending flow for about 4 h. The colour reagent was 0·25 per cent ninhydrin in acetone with 5 per cent collidine.

Using 16 and 32 μl amounts of the N bases solution with ninhydrin testing, a strong purple spot from ammonia was noted around R_f 0·0 and a weak purple spot around R_f 0·73–0·74. A 2 μl amount of 0·01 N nor-pseudoephedrine in a comparison test on an adjacent area of paper also showed

a spot at R_f 0·75. The volatile bases solution made from khat alone shows no colour reaction with ninhydrin around R_f 0·80. When 0·1 mg of amphetamine sulphate is added to the 20 g khat sample, a faint purple hue is additionally notable around R 0·80. When 2 μl amounts of 0·01 N amphetamine are used directly as a comparison test, a faint spot around the same R_f value is noted. The methyl red present in the distillate solution affords no confusion as it is visible before any use of reagent, at R_f 0·85.

Aqueous Extracts

Aqueous infusions containing the extract of 5 to 15 g of dried Catha leaves were used for some tentative therapeutic trials in man by Leloup.[39] These trials were inadequately reported but considerable astringent quality of such extracts was noted. Stockman[20, 21] reported swallowing infusions of 5 to 20 g of material without experiencing any definite feeling of stimulation. He remarked that a decoction made from 10 or 15 g of material with a cupful of milk had a somewhat pleasant liquorice-like taste.

A 10 g main lot of powder was mixed with 1000 ml of water and the mixture brought to a gentle boil for 10 min. The extract was pressed out and the residue washed to yield 1000 ml. The solution tasted bitter-sweet, was astringent and disagreeable to swallow in more than 50–100 ml portions at a time. Taken ice-cold, Alles was able to swallow and retain a total of 1000 ml. Gastro-intestinal discomfort and feelings of nausea were largely absent after 1 h. An increased tendency to move about and some feeling of increased alertness was felt to be present. At 3 h, there was no inclination to eat at the usual lunch time.

An attempt by Alles to take and retain 1000 ml of 1 : 50 extract without icing was not successful and only half of the solution was retained. When an iced solution in the same amount was taken more slowly the solution was retained in a following trial. Continuing gastrointestinal discomfort and anorexia lasted well over 8 h. Some increase in subjective alertness occurred and this lasted into the evening with better than usual vision and interest in reading.

20 g of main lot powder was also extracted with only 200 ml of water with gentle boiling for 4 h. The extract was then pressed out and the residue washed to give 200 ml of 1 : 10 extract. Such extract contained 0·66 mg per ml of tannin and was very bitter and astringent to taste. Well-iced it was swallowed by Alles in small portions. Considerable gastrointestinal discomfort resulted, but all was retained. After 1 h slight increase in subjective alertness was noted. Anorexia was noted and continued long after this period. Addition of 200 ml of skim milk derived from solution of 20 g of non-fat milk powder to 200 ml of 1 : 10 extract and cooling gave a more palatable solution to ingest. This did not cause any considerable

gastrointestinal discomfort. No significant changes in blood pressures or heart rates were noted during a 2-h supine test period and no considerable subjective effects were noted during this period. During the following ambulant period some anorexia was noted for a few hours and some increase in alertness, reading vision and interest.

Detannated Aqueous Extracts

The primary object of the present study was to identify and evaluate the pharmacological activities of aqueous extracts of Catha materials. It became necessary to obtain and work with concentrated extracts. Simple concentration by evaporation of the brown 1 : 10 aqueous extracts caused long continuing precipitations of some phlobaphenes and resulted in intensely black solutions. Beiter[19] had indicated that most of the tannin substances could be removed as insoluble lead salts.

Details of a method for preparing a 1 : 1 detannated aqueous extract suitable for various isolations and testings were developed. Following the chromatographic indications from this extract we were later able to fractionate and isolate two substances of notable pharmacological activity and correlate their amounts with the more notable pharmacological activities of aqueous khat extracts.

The 1 : 1 detannated extract also afforded a solution suitable for the estimation of the amounts of reducing sugars present in Catha. The Lane–Eynon volumetric method[40] indicated the presence of 1·4 per cent of total reducing sugars, calculated as a hexose. A considerable portion of the reducing sugars may be galactose, for the corresponding sugar alcohol dulcitol is present in considerable amounts in Catha materials.[41] We were also able to isolate dulcitol in the fractionation of the detannated extract. Some of the reducing material included in the reducing sugars estimation certainly included the ascorbic acid estimated to be present by Mustard[42] from the extent of reduction of a dichloroindophenol reagent.

Aside from solvent-extractable and Reineckate precipitable bases isolated in later fractionation work, our chromatographic studies of the detannated extract indicated the presence of considerable amounts and numbers of amino acids. Among those evident, lysine, γ-amino-butyric acid, phenylalanine and phenylserine were well identified by two dimensional chromatography.

Phenylalanine and phenylserine are of special interest because the results of Leete[37] established the biogenesis of *dextro*-nor-pseudoephedrine in Catha from phenylalanine.

The inorganic cation content of the initial 1 : 10 aqueous extract was little affected by the lead precipitation and the removal of excess lead ion as sulphate and finally as sulphide. Spectrographic analysis of the 1 : 1 detannated extract showed it to be about 0·18 N in K, 0·15 N in Mg, 0·04 N in Ca and 0·03 N in Na ions, with less than 0·0001 N Pb ion resultant from the detannating treatment. In processing from the 1 : 10 extract about 70 per cent of the Ca content has been removed.

250 g of main lot powder was gently boiled with 2500 ml of water for 1 h and the extract pressed off through cloth, then the residue washed with three 250-ml portions of water to give about 2500 ml of filtrate of about pH 5·6. The warm extract was treated with 50 g of basic lead acetate powder with thorough stirring for 30 min and the insoluble material filtered off and washed with three 75-ml portions of water to give about 2500 ml of filtrate of about pH 5·0. The filtrate containing lead was treated with 12·5 ml of 12 N sulphuric acid, and lead sulphate filtered off and washed with some water. The filtrate was evaporated to about 250 ml, then treated with hydrogen sulphide and again filtered. Some further evaporation, then addition of water, gave 250 ml of 1 : 1 detannated extract of about pH 4·0. Chromatography of 1, 2, 2 × 2 and 4 × 2 μl amounts of the 1 : 1 extract with freshly prepared 60 : 25 : 15 butanol–acetic acid–water solvent and using ninhydrin as the spot test reagent showed blue areas around R_f 0·72–0·74 with all these amounts of extract. Comparable intensities of colours are shown centring about the same R_f values with equal amounts of 0·01 N nor-pseudoephedrine sulphate.

Apart from the R_f 0·72–0·74 blue areas comparable to nor-pseudoephedrine, many ninhydrin positive areas are noted. The more obvious of these centre about R_f values of 0·03, 0·11, 0·20, 0·27, 0·36, 0·42 (yellow), 0·46, 0·57, 0·64. These are amino acids and a few of them were identified by two dimensional chromatography, using a 80 : 20 : 4 methanol–water–pyridine as the second solvent. Proline accounts for the yellow spot at R_f 0·42.

Alkalinization of the 1 : 1 extract to pH 10 with 18 N NaOH develops an odour of ammonia and a flocculent precipitate. The filtrate from this precipitate was extracted 5 times with equal volumes of chloroform–ether and the filtered extracts evaporated in room air gave an aqueous condensate which was adjusted to pH 5·0 and made up to a 20 : 1 concentration in terms of khat materials originally used. Chromatography of such a solution with 1 to 4 μl amounts and using either freshly prepared 60 : 25 : 15 butanol–acetic acid–water or 80 : 20 : 4 methanol–water–pyridine as the distributing solvents shows ninhydrin spots only in the same R_f area and

comparable in colour to tests with the same amounts of 0·1 N nor-pseudoephedrine sulphate. These areas also respond to testing with the aceto-acetylphenol reagent of Baker[34] which is specific for primary amines.

When chromatograms of 2, 2 × 2 and 4 × 2 μl amounts of 1 : 1 extract are tested with diluted Dragendorf reagent[38] none of the ninhydrin positive test areas are shown. An area around R_f 0·56 is shown, however, and the test intensity and R_f value correspond to comparable amounts of a 0·03 N choline sulphate solution.

Pressor and Depressor Activities of Detannated Extracts

Except for the notable contribution of the tannins to the pharmacology of Catha extracts, the effects of detannated extracts afford a full study of their pharmacological activities. When Catha materials are chewed and swallowed as amounts from the plants, the materials are subjected to effective aqueous extraction with the rate controlled by the consumer as long as the bulk of material remains in the mouth.

Our principal initial interest was to attempt a comparison of the pressor activities of detannated extracts with those resulting from standard amounts of *dextro*-nor-pseudoephedrine. The marked tachyphylaxis of the responses of successive dosages of this base related to ephedrine had been noted by all who had attempted its pressor study.[28, 43, 44] In consequence, no great precision in such comparisons can be expected.

Experiments with regard to pressor effect were first made with atropinized dogs under pentobarbital anaesthesia. An adequate dosage of the detannated extract caused a prompt, sharp, but transient, rise in blood pressure accompanied by corresponding stimulation of respiratory rate and amplitude, then a slowly increasing and relatively prolonged rise in blood pressure without notable changes in respiration. Injections of the chloroform–ether extractable bases in comparison showed only the slow rise in mean blood pressure without notable respiratory change. It thus became obvious that there was some active agent in the detannated extracts other than *dextro*-nor-pseudoephedrine and that this agent was capable of exerting nicotine-like pressor effects when injected following an adequate dosage of atropine.

When mecamylamine is given in an adequate dosage[45] to an atropinized dog, a subsequent injection of nicotine becomes largely ineffective in producing any pressor effect. The detannated

extract was found to be relatively undiminished in its ability to cause the sharp pressor effect with attendant respiratory stimulation. The secondary and more prolonged pressor effect of the detannated extracts was also not notably changed by mecamylamine pretreatment.

Our chromatographic studies had progressed to indicate that choline or a very similar compound was present in the detannated extracts in substantial amounts. When injections were made of the detannated extract into dogs without atropine pretreatment the presence of a sharply acting depressor component was evident, along with the prolonged pressor effect of the *dextro*-nor-pseudoephedrine present. The sharply active depressor component was not present among the chloroform–ether extractable bases. It was later found to be precipitable as a Reineckate salt from alkaline solutions.

A dog under anaesthesia with 40 mg/kg of sodium pentobarbital given intravenously, was also injected with 3×10^{-7} moles/kg intravenously of *l*-norepinephrine and *d*-nor-pseudoephedrine, then 0·01 and 0·03 ml/kg of 1 : 1 detannated khat extract was injected. As shown in Fig. 1, with the larger dosage a sharp rise in blood pressure accompanied by a sharp increase in respiration was noticeable. The secondary pressor effect approximately matches the effect of the 3×10^{-6} moles/kg of *d*-nor-pseudoephedrine given earlier.

Another animal to which 3×10^{-5} moles/kg of mecamylamine hydrochloride[45] had also been given was shown to be now non-reactive to 10^{-7} and 10^{-6} moles/kg of nicotine. However, even 0·01 ml/kg of 1 : 1 detannated extract was still found to cause its previous sharp rise in blood pressure with attendant sharp increase in respiration.

A dog under 40 mg/kg of sodium pentobarbital anaesthesia was first calibrated in response to 10^{-9} moles/kg of *l*-epinephrine and to 10^{-7} moles/kg of nicotine. In this animal 0·01 ml/kg of 1 : 1 detannated extract caused a small and prolonged pressor effect while 0·03 ml/kg caused a sharp depressor effect which was followed by a secondary rise in pressure. A transient inhibition of respiration was noted during the initial depressor effect. A similar depressor response was noted following the injection of 10^{-6} moles/kg of choline sulphate intravenously.

Motor Stimulant Activites of Detannated Extracts

The central stimulant activities of khat materials are of primary pharmacological interest in connection with its usage. The motor stimulant activity of ephedrine and amphetamine-like compounds

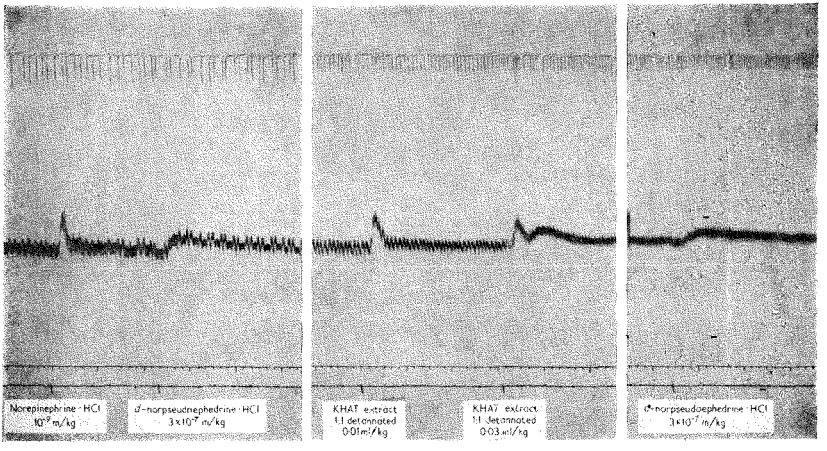


Fig. 1. Pressor effects of a 1 : 1 detannated extract

Dog, under 40 mg/kg of sodium pentobarbital anaesthesia and injected with 3×10^{-5} moles/kg of atropine sulphate. Blood pressure and the respiration tracings. Intravenous injections at 5-min intervals.

(a) 10^{-9} moles/kg of *l*-norepinephrine hydrochloride
(b) 3×10^{-7} moles/kg of *d*-nor-pseudoephedrine hydrochloride
(c) 0·01 ml/kg of 1 : 1 detannated khat extract
(d) 0·03 ml/kg of 1 : 1 detannated khat extract
(e) 3×10^{-7} moles/kg of *d*-nor-pseudoephedrine hydrochloride

were given some study in rats by Schulte and co-workers.[46] They found that 40 mg/kg subcutaneous doses of racemic norephedrine hydrochloride caused some increase in motor activity. Hoffman and co-workers[28] reported, without giving any details, that 50 mg/kg subcutaneously represents a minimal stimulating dosage of racemic nor-pseudoephedrine hydrochloride for mice. Von Brücke[26] had found 100 mg/kg *dextro*-nor-pseudoephedrine sulphate subcutaneously, but not 50 mg/kg, was required minimally to stimulate motor activity in mice.

By systematic study of motor activity of mice under well controlled conditions this pharmacological activity of 1 : 1 detannated khat extracts was given quantitative valuation on a statistical basis, in comparison with the activity of *dextro*-nor-pseudoephedrine hydrochloride. It was found that the motor stimulant activity in mice of 10 ml/kg of the detannated extract was closely matched by the injection of about 1·35 mg/kg of *dextro*-nor-pseudoephedrine, calculated as the base.

Motor activity of mice was studied in Actophotometer units as made by Metro Industries of New York. These units are cylindrical solid wall cages with 12 holes equally spaced around the walls and near the base, above a floor adjustable in height. Half of these holes have infrared light sources behind them and the oppositely placed hole carries a photo-cell. Each time an animal moves across a light beam one count is recorded on a digital counter. The studies were made in a temperature controlled room at 26°.

Swiss white mice of 20–30 g weight were kept in the room for 12 h or more before use. Then to condition them to the testing they were injected intraperitoneally with 0·10 ml of normal saline solution and placed in a separate actophotometer unit for 75 min. They were then injected with about 0·10 ml of test solution and again placed in the unit for a further 75 min. Counts were recorded for the 45 to 75-min period. When saline was used for test solution, counts ranging from 0 to 425 for the 30-min period were noted among 90 control animals. Test dosages of 0·037, 0·055, 0·082, 0·123, and 0·184 mmoles per kg of *dextro*-nor-pseudoephedrine were used with 8 animals per dose. This represents a dosage range of 7 to 35 mg/kg of *dextro*-nor-pseudoephedrine hydrochloride.

A positive stimulant response was concluded for each animal that exceeded a count of 500 for the 45 to 75-min interval test period. From such data a dose–effect curve was graphed following the details of the statistical method of Litchfield and Wilcoxon[47] and the effective dose ED_{50} with 19/20 confidence limits was found in the experiment described to be 0·093 (0·054–0·160) mmoles or 14·0 (8·15–24·2) mg of *dextro*-nor-pseudoephedrine base per kg.

A corresponding procedure was followed with the use of 5 doses of 1 : 1 detannated extract also with 8 animals per dose. From the data there was graphed another dose–effect curve and the ED_{50} was found to be 10·8 (9·15–12·75) ml per kg. As some local irritation was noted to result from the injection of the extract it was adjusted from pH 4·9 to 7·0 and retested. The ED_{50} was found on this retest to be 10·4 (8·95–12·08) base per ml of the 1 : 1 detannated extract. From this motor stimulant data it can be calculated that the presence of 1·35 (0·91–2·00) mg/ml of *dextro*-nor-pseudoephedrine base per ml of the extract could account for the motor stimulant activity shown.

Inhibitor and Stimulant Activities on Intestinal Muscle

Both aqueous and detannated khat extracts were found to have marked relaxant properties when added to the bath of an isolated rabbit ileum preparation. At least half of the inhibitory effect shown by a detannated extract was found to be due to the contained inorganic ions derived from the plant material. Not more than one-twentieth of the inhibitory activity of such an extract can be ascribed to the *dextro*-nor-pseudoephedrine content of the detannated extract.

The initial aqueous extract of the dry khat material was often notable as compared with a detannated extract in producing a secondary increase in tone concomitant with a marked decrease in amplitude of movement of the intestinal muscle. Such an effect is also shown by 1 per cent solutions of pharmacopoeil tannin added to the test bath. This indicates that the similar tannin content of aqueous Catha extracts may contribute substantially, along with the inorganic constituents, to the considerable gastrointestinal effects when *Catha* or its simple aqueous extracts, are consumed by man.

Rabbit ileum strips suspended in a medium of 0·9 per cent NaCl, 0·042 per cent KCl, 0·018 per cent $CaCl_2$, 0·015 per cent $NaHCO_3$ and 0·10 per cent glucose at 37° were used for study.[48,49] The bath used had a working volume of 10 ml and when 0·1 ml of 1 : 1 detannated extract was added a substantial relaxation resulted with decrease in amplitude of movement. When some of the extract was ashed, then neutralized and taken up in the same volume of water, 0·2 ml of the 1 : 1 ash solution evoked a comparable response due to its content of Mg and possibly some other inorganic ions. When 0·1 ml of 20 : 1 solution of pH 10 chloroform–ether extractable bases of the detannated extract was tested in water solution a slight contraction followed by relaxation was noted that was

comparable to the relaxant effect of 0·1 ml of 1 : 1 detannated extract. The effect of the 20 : 1 extractable bases was also matched by the effect of 0·1 ml of 0·25 N *dextro*-nor-pseudoephedrine hydrochloride.

As with the racemic norephedrine,[50] the response of individual ileum strips with a particular concentration of such a substance, may result in an increase or decrease of tone or a mixture of both effects. The most precise matching of any of these effects of 0·1 ml of 20 : 1 total extractable bases occurred with 0·06 ml of 0·05 N *dextro*-nor-pseudoephedrine. This indicated the 20 : 1 solution to be about 0·15 N in content of this base. The detannated extract from which it was extracted would therefore be about 0·0075 N or contain about 1·1 mg of *dextro*-nor-pseudoephedrine base per ml.

A 1 : 1 aqueous extract added before the detannating treatment caused marked relaxant response with 1·0 ml and this effect was similar to that of 0·1 ml of 1 : 1 detannated extract. However, as shown in Fig. 2, a secondary increase in tone may also be noted with the aqueous extract and a similar response is shown with 1·0 ml of a 1 per cent solution of tannin, U.S.P., which contains tannin comparable in amount to that present in the 1 : 10 aqueous khat extract.

Fractionation of Detannated Extract

After trying fractionating procedures on detannated extracts, it appeared best to first concentrate to one-fifth of the original volume. This resulted in good separation of the dulcitol present[41] in an amount of 0·9 per cent by weight of the Catha material used with not more than an additional 0·6 per cent remaining dissolved in the 200 ml 5 : 1 concentrated solution.[51] The isolated material was identified in part by its crystal appearances from water solution.[52] Plouvier[41] had found dulcitol present in all *Celastraceae* species examined but offered no estimation of its total amounts.

Alkalinization to pH 12 gave coagulation of precipitated inorganic hydroxides and passing in carbon dioxide to pH 10 added some insoluble carbonates to the precipitation. The pH 10–12 precipitate readily dissolved in a neutralizing amount of sulphuric acid and spectrographic analysis of the ash of this solution showed it contained magnesium as the principle cation. Chromatography showed only trace amounts of ninhydrin positive substances, not in excess of amounts expected to be absorbed by the bulky colloidal precipitation. Biological testing of the 2 : 1 solution showed it to be as active as a 1 : 1 detannated extract in causing a

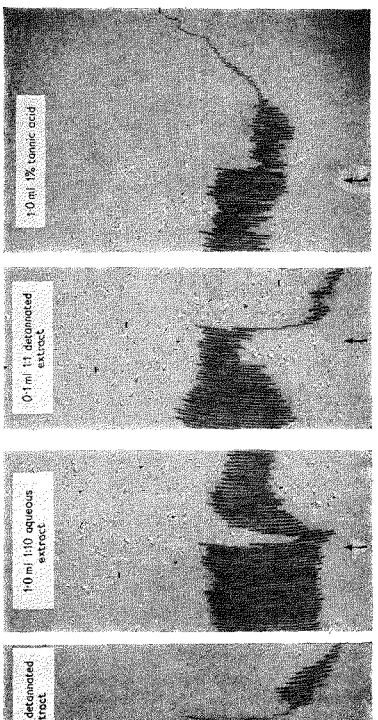


Fig. 2. Effects on isolated rabbit ileum suspended in 10 ml of modified Locke solution oxygenated at 37°.
(a) 0·1 ml of 1 : 1 detannated khat extract
(b) 1·0 ml of 1 : 10 aqueous khat extract
(c) 0·1 ml of 1 : 1 detannated khat extract
(d) 1·0 ml of 1 : 100 tannic acid U.S.P.

notable effect on rabbit intestine. This was due to the magnesium ion content, and possibly some other inorganic ions, for identical activity was shown by a solution of the ash of the 2 : 1 solution. The magnesium content of the detannated extract can account substantially for its relaxing effect upon the intestine.

Extraction of chloroform–ether extractable base material almost completely removed the *dextro*-nor-pseudoephedrine present. This base alone was again isolated as its optically pure sulphate and further identified by conversion into its dibenzofuran-2-sulphonate. The *dextro*-nor-pseudoephedrine sulphate isolated corresponds with amounts indicated as present by the chemical estimations of total bases and by the bioassay results with the 1 : 1 detannated extract.

After removal of chloroform–ether extractable bases, the quaternary ammonium type compounds precipitable as Reineckates under alkaline conditions[53] were taken out. Chromatography of the pH 10 Reineckate indicated choline and only choline to be present, by testing with Dragendorf reagent. The crude Reineckate was converted into the well crystallized dipicramate by the method of Ackerman.[54] Calculated from the recrystallized choline dipicramate, the choline content of the Catha material is about 0·05 per cent calculated as its hydroxide or 0·065 per cent calculated as sulphate. This would put about 0·0042 N in the 1 : 1 detannated extract, comparable to the amount estimated from its depressor and pressor activities.

The residual solution after removal of the pH 10–12 precipitate, and the extractable bases, and finally the Reineckates at pH 10, was freed of Reineckate ion and given some chromatographical and biological test study. Slight amounts of nor-pseudoephedrine-like material still appeared to be present in the residual solution, but its amount was not more than 5 per cent of that which had been isolated.

Detannated extracts from four 250-g batches of main lot Catha were combined and evaporated in air to 100 ml, then allowed to stand several days to complete separation of insoluble materials. These were filtered off and the filtrate was set aside. The insoluble material was extracted with small volumes of hot water, which gave on evaporation 7·8 g of coarse white crystals, m.p. 184–188°, and a second crop of 2·3 g which melted, but not completely, at 188°. These crops were combined and recrystallized from 20 ml of hot water to yield 9·0 g of dulcitol m.p. 186–188°.[41,51]

The 200 ml of 5 : 1 solution after filtration was alkalinized to pH 12·2 with 30 ml of 18 N NaOH. The pasty precipitate formed could not be filtered until 200 ml of water was added and adjusted to pH 10·6. The precipitate was filtered off and washed with water to yield 500 ml of filtrate. The pH 10–12 precipitate was warmed with 350 ml of 0·50 N H_2SO_4 to give a solution at pH 5·8 which was filtered and made up to 500 ml of a 2 : 1 solution. Ashing of this solution gave 2·16 per cent ash that gave spectrographic analyses of 16 per cent Mg, 3 per cent each of Na and K, and about 0·1 per cent amounts of Fe and Al ions. This would correspond to the solution having been 0·14 N in Mg ion. Chromatography of 5 μl amounts with BuOH : AcOH : H_2O solvent and ninhydrin testing showed traces of blue–violet spots corresponding to some of the amino acid material present in detannated extracts and a slight hue at R_f 0·73 corresponding to that of nor-pseudoephedrine in threshold dilutions. No spots responded to Dragendorf reagent testing.

The 500 ml filtrate from the pH 10–12 precipitate was then treated with CO_2 without further precipitation. With the solution at pH 10·2 it was extracted with six successive portions each of 500 ml of chloroform–ether (1 : 3). These extracts were filtered through paper, then evaporated to a condensed water residue which required 11·3 ml of 0·50 N H_2SO_4 to adjust to pH 5·8. Further evaporation to 5 ml gave a semi-crystalline mass and addition of 150 ml of acetone separated 1·18 g of solid material. This dissolved with 10 ml of hot water and was treated with some charcoal then filtered clear.

Addition of 50 ml of ethanol, then 150 ml of ether gave a flaky white crystal sulphate weighing 1·03 g, m.p. 282–284° (d.), about 2° less than a comparison sample of synthetic *dextro*-nor-pseudoephedrine sulphate. A 0·200 g sample made up to 10·0 ml with water in a dm tube rotated D light −0·80° at 26°. This corresponded exactly with the rotation of a synthetic sample. By pouring the sample from the optical determinations each into 5 ml of hot 0·02 N dibenzofuran-2-sulphonic acid there was obtained 0·27 g of isolated dibenzofuran-2-sulphonate, m.p. 218–220°; as compared with the synthetic salt, m.p. 220–221°.

The residual acetone solution from the separation of solid sulphate from the 1000 g of plant material was evaporated and the residue taken up with 5 ml of water and filtered clear. Chromatography of this 200 : 1 solution in 4 × 2 μl amounts primarily showed the presence of some additional nor-pseudoephedrine base along with traces of the amino acid materials of detannated extracts.

The solution after chloroform–ether extractions was at pH 10·2 and the solvents remaining in the water were blown off with air to a volume of 400 ml. This solution, at pH 10, was treated with 200 ml of 5 per cent ammonium reineckate solution in methanol. After standing overnight the precipitate was filtered off and washed with some water. It was then treated with 200 ml of acetone and the solution filtered clear, then evaporated to yield 2·6 g of red flaky solid. This product was dissolved completely in 150 ml of acetone with 30 ml of water, then treated with 200 ml

of hot Ag_2SO_4 solution. The silver reineckate precipitate was filtered off, the filtrate treated with H_2S to remove excess Ag^+, then the Ag_2S filtered off. Evaporation to 25 ml gave a 40 : 1 solution of pH 10 reineckate.

Chromatography of this solution in 2 to 4×2 µl amounts with the BuOH : AcOH : H_2O solvent and Dragendorf reagent testing gave two areas of test, around R_f 0·48 and around 0·20. The first area corresponds to that given by a neutral solution of 0·1 N choline sulphate in 2 µl amounts. The R_f 0·20 spot is an acid form of choline due to the acidity of the solution. No other quaternary ammonium bases were found present. When 20 ml of this solution was neutralized to pH 7 and 40 ml of 0·1 N Mg dipicramate[53] was added a heavy precipitate resulted. This was filtered off and dried to 1·7 g of crude choline dipicramate which readily recrystallized from a little hot water to give 1·65 g, m.p. 232–234°, of pure choline dipicramate. This corresponds to 4·2 milliequivalents of choline per kg of Catha material or 0·042 N in the detannated extract. The filtrate from the reineckate precipitation was partially evaporated. Adding 7·0 ml of 12 N H_2SO_4 gave pH 7·0, and 600 ml of hot 1 per cent solution of Ag_2SO_4 removed all reineckate ion. The filtrate from the Ag reineckate was evaporated to 300 ml, treated with H_2S, filtered clear and evaporated. The residue was made up to 500 ml of 2 : 1 residual solution for testing in man.

Chromatography showed the presence of the starting amino acids but almost no nor-pseudoephedrine. A trace amount was evident with 6×2 µl, but not more than ¼ of that in 2 µl of 1 : 1 detannated extract. There was no indication of any choline being present in the residual solution.

Subjective Effects in Man

While in Ethiopia to obtain samples for study, Alles purchased an acara of fresh Kuda material in the market. It consisted of two bunches each of 14 branch ends with leaves and wrapped into large leaves of another plant to retain moisture. During ½ h the edible leaves and branch tips of nine branch ends were chewed and swallowed. The weight of the nine plant pieces was about 300 g and two-thirds was eaten as being adequately succulent. The taking of this caused notable alertness and resulted in unusually animated talking for 2 h with some visitors. Definitive subjective stimulation continued for 4 to 6 h, comparable to the effects of 10 mg of racemic amphetamine sulphate in the same subject.[48] The gastrointestinal feeling resultant seemed agreeable, not unlike that following the consumption of the bitter coffee or quinine water in these same tropical areas. The 200 g of fresh material consumed would correspond to about 60 g of air-dried material.

Later the experience of Alles was that the swallowing of aqueous

extract from as little as 20 g of dry Catha material caused some degree of disagreeable gastrointestinal effects. However, after the tannins are removed from dry material extracts the aqueous extract becomes satisfactory to swallow in amounts adequate for notable central nervous stimulation. Detannated extracts in amounts corresponding to 20 g of dry material when taken by mouth usually cause notable effects but after amounts corresponding to 40 g are taken the subjective effects are more clearly evident.

Doses of 20 mg of *dextro*-nor-pseudoephedrine hydrochloride caused only minimal subjective effects, of the order of the effects of 20 ml of 1 : 1 detannated extract. Following doses of 40 and 60 mg of *dextro*-nor-pseudoephedrine salt, subjective effects were more evident. The effects of the 60 mg dose were most comparable to those following the taking of 40 ml of 1 : 1 detannated extract, derived from 40 g of dry main lot of *Catha* material.

The gastrointestinal effects resulting from the taking of the total extractable bases that were derived from 20 ml of detannated extract were minimal as compared with those following this amount of detannated extract. The residual solution after removal of all bases extractable by chloroform–ether and butanol was found to be inactive when taken in amounts corresponding to 80 g of dry plant material. Further experiments with the residual solution after separation of the *dextro*-nor-pseudoephedrine and choline as described showed the residual substances to have no notable effect even in doses corresponding to as much as 160 g of dry Catha material. Thus more than seven-eighths of the initial activity of the detannated extract had been removed.

Experiments were carried out on the same individual at different times under controlled laboratory conditions. The subject remained supine for two or more hours during the observations of blood pressure, heart rate and electrocardiogram. The materials were taken along with 200 ml of water about 2 h after a light morning meal and lunch was postponed until the conclusion of all observations of possible circulatory effects.

Experiments were with 20 ml of 1 : 1 detannated extract. Increases of 10–16 mm in systolic and 6–12 mm in diastolic pressures with a change of 4–6 pulse per min were noted but are not definitely outside normal variations. Some gastrointestinal discomfort and loss of appetite occurred. Opening of the nasal airway and dryness of the throat was evident. Increased awareness of environment and of heart rate was noted in one trial.

A trial of 40 ml was made and within 1 h the nasal airway was more open and there was increased alertness. Restlessness and feelings of increased muscular tensions were noted. The observer noted increased eye and other body movements. After 90 min there was increased attention to internal and external phenomena and breathing was slowed and deepened. Increased alertness and talking continued for about 12 h and sleeping that night seemed light but was felt to be adequate the next morning. Anorexia was evident for 10–12 h.

With doses of 40 mg and 60 mg of *dextro*-nor-pseudoephedrine hydrochloride notable subjective stimulant effects resulted and some circulatory effects were noted. With the higher dose, a 30 mm increase in systolic and 12 mm increase in diastolic pressures was noted 3 h after the administration of the test dose. Decreases in heart rate of as much as 6 beats per min were noted at this time. The nose was opened, the nose and mouth felt dry and respiration was slowed, with deepening. Marked alertness to external and internal phenomena resulted. Attention to and good vision for reading remained increased for 12 h but sleep thereafter appeared usual. Feelings of gastrointestinal fullness and anorexia were persistent.

Study was made of the effects of the solution residual from complete extraction with both chloroform–ether mixture and butanol of detannated extract at pH 10. The residual solution made neutral as a 5 : 1 concentrate was taken in 8 and 16 ml amounts. Only slight changes in blood pressures without any changes in subjective alertness were noted. Another solution, residual from removal of extractable bases and followed by the removal of reineckate precipitable substances, was taken in amounts that corresponded to 80 and 160 ml amounts of 1 : 1 detannated extract without any notable circulatory changes or central effects being observed.

Discussion

Our estimations of total extractable bases agree well with those reported by Paris and Moyse[32, 33] when possible differences in method and mode of calculation are considered. Our isolation of about 0·1 per cent *dextro*-nor-pseudoephedrine as sulphate in its optically pure form is somewhat higher than the quantities of crude total base sulphate isolated by Beiter.[19] The exceptional optical purity of the material readily isolated indicates the absence of substantial amounts of any extractable bases other than *dextro*-nor-pseudoephedrine. Specific examinations for amphetamine isomers showed them not to be present in significant amounts, from the standpoint of making a contribution to the stimulant activities of the plant materials, as suggested by Von Brücke.[26, 27] Chromatographic studies of the total extractable

base fraction did not evidence any considerable amount of other norephedrine or ephedrine isomers, or indeed of any other bases responding to general test reagents for nitrogenous bases. Tests with reagents notably sensitive to imidazole, indole, or phenol type bases showed their absence.

Roughly quantitative comparisons in man of the central stimulant aspects of the action of khat plant material, its aqueous extracts, and its detannated extracts, gave results that corresponded to the amounts of *dextro*-nor-pseudoephedrine isolated. When the effects of the total extractable bases were compared with comparable amounts of detannated extract in man there was a correspondence in effects. The solution residual from the removal of extractable bases was inactive with even an eight times increased dosage.

Precise quantitative evaluations in mice of the motor stimulant activities of detannated extracts in comparison with *dextro*-nor-pseudoephedrine showed that such central activity could be well accounted for by the amounts of this base present.

Studies of the pressor and depressor activities of detannated and other khat extracts when injected intravenously into dogs indicated the presence of considerable amounts of some vasoactive material other than *dextro*-nor-pseudoephedrine. It was found that this was a non-extractable base that could be precipitated as an insoluble reineckate. This material was finally isolated as a dipicramate and this product established as being pure choline dipicramate. Chromatographic evidences during its isolation did not show the presence of any substantial amount of any other quaternary ammonium base. The amounts of choline that will be absorbed from oral ingestions of khat materials can only be of some nutritional significance, for dosages as large as 10 g of a choline salt can be taken orally by man without obvious pharmacodynamic response.[55]

The marked astringent quality of aqueous extracts of khat materials is related to the most notable gastrointestinal effects resulting from administration of these materials. These effects are minimized in the consumption by the slow extraction from chewed green plant material and are most notable when concentrated aqueous extracts of dried material are swallowed within a short period of time. These effects are made less evident by

admixture of the plant extracts with milk and appear to be largely due to the quantities of extractable tannins found in the plant material.

When the action of extracts is studied upon isolated ileum preparations it becomes evident that the inorganic ions present in such extracts may also produce very substantial inhibitory actions upon gastrointestinal function. Such actions are many times greater than can be ascribed to the actions of the contained *dextro*-nor-pseudoephedrine. It would appear that magnesium plays a large rôle among the inorganic ions present.

Aside from the action of the tannins present in khat materials upon the gastrointestinal tract, it would seem probable that the tannins may also play a substantial rôle in determining the subcutaneous lethal dose when extracts are injected into animals.[33] The toxicity of aqueous khat extracts under such conditions was found to be less than that of tea or maté extracts.[33] The extractable bases of khat materials were found not to be comparably toxic to those containing the tannins even with a four times greater dosage based on the amount of plant material used.[33]

Satisfactory classification of the phlobaphene-producing tannins such as those present in aqueous khat extracts, is most difficult.[56] Further, the relative pharmacological properties of tannin materials has been found to vary substantially among different types.[57] However, there is agreement that tannins can generally be quite readily absorbed from the gastrointestinal tract.[58] When absorbed at low levels of dosage, tannins may enhance and prolong the effects of epinephrine in its actions in the body.[59, 60] However, in large doses the tannins can be very substantially toxic to animals and lead to liver and adrenal damage.[61, 62]

Summary. *Catha edulis*, grown and selected in Ethiopia as suitable khat material, contains about 0·1% of *dextro*-nor-pseudoephedrine on the basis of air-dried material. This base occurs without any notable admixture of its isomers or of ephedrine isomers. Amphetamine isomers or other extractable bases are not present in pharmacologically significant amounts. The central stimulant quality and quantity of extracts of such material can be well accounted for by the amount of *dextro*-nor-pseudoephedrine therein found present. About 0·05 per cent of choline is also present and contributes no significant pharmacological effect from oral ingestion of these materials. Inorganic ions, particularly magnesium ion, are present in amounts sufficient to cause local gastrointestinal effects. Phlobaphene-

producing tannins are present in sufficient amounts within the consumed dosage range to produce marked gastrointestinal effects, causing responses on up to nausea and vomiting. These tannins also may produce effects systemically when adequate amounts are consumed, acutely or chronically.

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