Investigation of the Fixed Oil from *Ipomoea digitata* Tubers

By S. S. MISHRA, J. P. TEWARI, and M. A. MATIN

The fixed oil forming 1.3 per cent of the tubers of *Ipomoea digitata* Linn. contains palmitic (8.15 per cent), oleic (60.10 per cent), linoleic (19.38 per cent), and linolenic acids (1.11 per cent) in mixed acid fraction.

IPOMOEA DIGITATA Linn. (family *Convolvulaceae*), known as Vidari Kand in Hindi, is a common indigenous drug used by Ayurvedic practitioners as a tonic, aphrodisiac, and galactogogue. It is found throughout tropical Asia and is very common in the northern marshy areas. It is a perennial creeper having tubers weighing up to 3 Kg. (1, 2).

Using a crude fraction isolated from the tubers of this plant, Mishra and Dutta (3) reported the presence of pressor activity. Tewari and co-workers (4) reported the presence of a phytosterol, a golden-yellow fixed oil, a neutral compound (m.p. 72°), and β-sitosterol in the nonsaponifiable matter of the oil. A glycoside, tentatively named paniculatin, also has been isolated from the tubers of this plant. Paniculatin raises blood pressure, stimulates respiration, and constricts smooth muscles like those of the gut, uterus, bronchus, and blood vessels (5).

The above observations prompted a further investigation of this plant. The isolation and characterization of the fixed oil have been described in this paper. The oil was obtained during the course of defatting the crushed powder for the isolation of paniculatin.

Several other seed fats of the family *Convolvulaceae* were analyzed earlier (6, 7). Some species of ipomoea have also been examined by Kassner (8). He states that oils from these species have similar physical and chemical properties and are similar to one another. The fatty acid composition of *I. digitata* Linn. tuber oil resembles markedly the composition of *I. muricata* (7) seed oil; it differs from *I. muricata* seed fat in behenic acid content (3.78%), as reported by Kelkar *et al.* (6). Behenic acid was found to be absent in the seed fat of *I. muricata* (7). The isolation of behenic acid by Kelkar *et al.* might probably be due to the fact that proper care did not seem to have been taken to free the oil from the glucosidic resin which invariably accompanied it. According to Agrawal and Dutt (9), the oil from *Cuscuta reflexa* Roxb. (family *Convolvulaceae*) does not contain behenic acid. Attempts also to isolate and identify behenic acid from the tuber oil of *I. digitata* Linn. were unsuccessful.

The pharmacological investigation of the oil is in progress.

EXPERIMENTAL

Sliced tubers of the plant were procured and air-dried. After establishing the pharmacognosy, these were reduced to a fine powder (No. 40 mesh) in a disintegrater. The authenticated specimen of the material investigated has been made and deposited for future reference.

The crushed tubers on petroleum ether (b.p. 40–60°) extraction in a Soxhlet apparatus gave a resinous oil, which then was purified with animal charcoal and Fuller's earth. Then the oil was passed over neutral Brockman's alumina and eluted with ether. On removing the solvent, a golden yellow oil was obtained in 1.3% yield having the following characteristics: specific gravity at 30°, 0.9120; refractive index at 30°, 1.4765; saponification value, 191.5; iodine value, 85.26; nonsaponifiable matter 1.10%; R.M. value, nil; acid value, 1.7. The oil had the peculiar odor of the powdered drug itself.

Saponification of 100 Gm. of the oil was performed with 0.5 N alcoholic potassium hydroxide solution, and the unsaponifiable matter was removed by the B.P. method (10). The mixed acids were liberated and showed the following constants: neutralization value 197.4; iodine value, 102.3; consistancy, semisolid.

The mixed acids then were separated into solid and liquid acids by the modified Twitchell's lead-salt alcohol method (11). The percentage and chemical constants of solid and liquid acids are given, respectively: solid acid fraction, yield 20.11%; neutralization value, 202.6; iodine value, 1.02; liquid acid fraction, yield 79.89%; neutralization value, 190.8; iodine value, 130.85.

The liquid acid gave a positive Elidian test, an indication of the presence of oleic acid.

Quantitative Estimation of Liquid Acids Fraction. —The constituents of the liquid acids fraction were estimated by Jamieson and Boughmann's (12) modified bromination method, and the results are given in Table I.

The constituent acid percentages in the liquid acids fraction also were estimated by the methyl ester method. The results were in close agreement with the bromination method.

The liberated fatty acids of each fraction of methyl esters were identified with the aid of paper chromatography. Whatman No. 1 paper strips were impregnated with liquid paraffin in petroleum ether (60–80°) (10% w/v). The fatty acids were dissolved in acetone, so that 35 mg. of the acids present in 25 ml. of solution, and applied to the paper followed by development with 90% acetic acid. After drying, the chromatograms were treated

TABLE I.—COMPONENT FATTY ACIDS IN THE LIQUID ACIDS FRACTION OF *I. digitata* LINN. TUBER OIL[a]

Acids	% in Liquid Acids	% in Mixed Acids
Oleic	74.34	59.39
Linoleic	24.25	19.38
Linolenic	1.51	1.11

[a] Results of three analyses.

TABLE II.—FRACTIONATION OF THE METHYL ESTER OF SOLID FATTY ACIDS OF *I. digitata* LINN. TUBER OIL

Fraction	Head Temp., °C.	Wt., Gm.
S_1	165–168	4.525
S_2	168–171	3.234
S_3	171–174	3.546
S_4	175–179	2.610
S_5	Residue	1.825

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TABLE III.—COMPONENT FATTY ACIDS IN THE SOLID ACIDS FRACTION OF *I. digitata* LINN. TUBER OIL.[a]

Acid	% in Solid Acids	% in Mixed Acids
Palmitic	40·52	8·15
Stearic	55·95	11·25
Oleic	3·53	0·71

[a] Results of three analyses.

TABLE IV.—COMPOSITION OF THE SOLID, LIQUID, AND MIXED ACIDS FRACTIONS OF *I. digitata* LINN. TUBER OIL

Acid	% in Solid Acids Fraction	% in Liquid Acids Fraction	% in Mixed Acids Fraction
Palmitic	40·52	...	8·15
Stearic	55·95	...	11·25
Oleic	3·53	74·34	60·10
Linoleic	...	24·25	19·38
Linolenic	...	1·51	1·11

with copper acetate and potassium ferrocyanide. The acids appeared as brown-red copper complexes.

Quantitative Estimation of Solid Acids Fraction.— The solid acids fraction was converted into methyl esters by the Fischer and Spier method, and 15.74 Gm. of this methyl ester was distilled under reduced

pressure (4 mm.). The results are shown in Table II.

Saponification and iodine values of each fraction were determined, and the liberated acids from each fraction were identified by paper chromatography. The amount of each acid in different fractions was calculated from these values. The percentage of various acids in the solid acids and in the mixed acids are listed in Table III.

The total percentage of various fatty acids in the solid, liquid, and mixed acids fractions of *I. digitata* Linn. tuber oil are listed in Table IV.

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Mechanism of Degradation of Penicillin G in Acidic Solution

By MICHAEL A. SCHWARTZ

By analysis of data available in the literature of the degradation of penicillin G in acidic aqueous solution, it is shown that two reactions are involved. One is the acid catalyzed hydrolysis of the undissociated penicillin molecule to form penilloic acid. The second is rearrangement of the penicillin ion following attack by proton or the kinetically equivalent uncatalyzed reaction of undissociated penicillin to form penicillenic acid which rearranges to penillic acid. The significance of this mechanism is discussed.

THE INSTABILITY of penicillin G (I) in acidic aqueous solution has been an important consideration in the design of dosage forms of the antibiotic. It is well known that penicillin decomposes in acidic aqueous solution to form both penillic acid (V) and penilloic acid (III) with a maximum yield of the former at pH 2–3 (1). It is also known that penicillenic acid (IV) is formed in acid solution and subsequently decomposes relatively rapidly (2). It may be detected by its characteristic ultraviolet absorption spectrum with maximum at 320 mμ. Recently, it has been proposed that penicillenic acid is responsible for penicillin allergy (3, 4). In the present work it is shown by mathematical analysis of data available in the literature that penicillenic acid, and subsequently penillic acid, arise from reaction of penicillin ion and penilloic acid from undissociated penicillin.

TREATMENT OF DATA

The data used in this work were from a report by Krejci (5). He determined rate constants for both over-all rate of loss of penicillin (k_P) and rate of formation of penicillenic acid (k_E) by means of polarography. Table I presents these data. The over-all rate constants were resolved into rate constants for proton catalyzed degradation of undissociated penicillin and penicillin ion by the method previously described for phenethicillin (6). The pKa of penicillin G was taken as 2.7 (7), and the plot of k_P/H^+ against fraction penicillin dissociated (f_{A^-}) is shown in Fig. 1. From the intercept and slope, the rate constants k_{HA} and k_{A^-} were evaluated as 2.6 and 10.8, respectively. The over-all equation for acid degradation of penicillin G may then be represented by

$$k_P = 2.6 \,[H^+]\,[f_{HA}] + 10.8\,[H^+]\,[f_{A^-}]$$

or at a single pH, since f_{HA} and f_{A^-} are functions only of pH,

$$k_P = k_1 + k_2$$