bound to Myosin B profoundly affects the isotherm in all regions.

### LITERATURE CITED

Bradley, S., J. Chem. Soc. 1467 (1936).

- Brunauer, S., Emmett, P., Teller, E., J. Amer. Chem. Soc. 60, 309 (1938). Bull, H., J. Amer. Chem. Soc. 66, 1499 (1944).
- Bull, H., Breese, K., Arch. Biochem. Biophys. 137, 299 (1970). Cassie, A., Trans. Faraday Soc. 41, 450 (1945).
- Gal, S., Bankay, D., J. Food Sci. 36, 800 (1971).
- Kanagy, J., Cassel, J., J. Amer. Leather Chem. Ass. 52, 248 (1957).

(1957).
Kapsalis, J., Wolf, M., Driver, M., Henick, A., Proc. Res. Conf., Res. Counc. Amer. Meat Inst. Found. Univ. Chicago 73 (1964).
Kapsalis, J., Technical Report AD 655488, Defense Documenta-tion Center, Cameron Station, Alexandria, Va., 1969.
Kapsalis, J., Walker, J., Wolf, M., J. Texture Stud. 1, 464 (1970).
Leeder, J., Watt, L., J. Phys. Chem. 69, 3280 (1965).

Ling, G., Ann. N. Y. Acad. Sci. 125, 401 (1965). Ling, G., Ann. N. T. Acad. Sci. 123, 401 (1966). Ling, G., Nagendank, W., Physiol. Chem. Phys. 2, 15 (1970). Mellon, S., J. Amer. Chem. Soc. 69, 827 (1947). Mellon, S., Hoover, E., J. Amer. Chem. Soc. 72, 2562 (1950). Morita, F., Tonomura, Y., J. Amer. Chem. Soc. 82, 5172 (1960). Palnitkar, M., Heldman, D., J. Food Sci. 36, 1015 (1971). Daviling, L. J. Amer. Soc. 67, 555 (1015). Pauling, L., J. Amer. Chem. Soc. 67, 555 (1945). Perry, S., Biochem. J. 55, 144 (1953). Salwin, H., Food Technol. 13, 594 (1959). Salwin, H., Food Technol. 17, 1114 (1963). Wolf, M., Walker, J., Kapsalis, J., J. Agr. Food Chem. 20, 1073 (1972).

Received for review January 24, 1973. Accepted July 16, 1973. This paper reports research undertaken at the U. S. Army Natick (Mass.) Laboratories and has been assigned No. TP-1325 in the series of papers approved for publication. The findings in this re-port are not to be construed as an official Department of the Army position.

## Relationship of Penetrometer Readings on Raw Beef with Cooked Tenderness

Deane G. Galloway, Justin M. Tuomy, and Larry C. Hinnergardt\*

This study was designed to evaluate the use of a simple penetrometer test to predict cooked beef tenderness from the force required to penetrate the raw beef sample. In addition, several factors which could bear on tenderness, such as waterholding capacity and fat content, were evaluated with the penetrometer readings by multiple regression techniques. An Allo-Kramer shear press was modified to function as a penetrometer by replacing the standard shear compression cell and shearing blades with a plate containing five needles. Simple linear correlation coefficients between the maximum penetration force in the raw

The tenderness of meat is generally regarded as its single most important quality and there is a very large body of literature concerning tenderness in all its aspects, including methods for determining and predicting it. Mechanical methods such as the Lee-Kramer shear press have been correlated with tenderness of cooked meats as determined by taste panels, but until recently only subjective methods such as USDA grade or marbling have been used to predict how tender a piece of raw meat will be when it is cooked.

Until recently, attempts to correlate mechanical tenderness ratings of raw meat with the tenderness of the same meat when cooked have been disappointing. Using the Warner-Bratzler shear, Warner (1928), Black et al. (1931), McBee and Naumann (1959), and Carpenter et al. (1965) found no significant correlations between raw and cooked meat. Informal work at these laboratories has shown the same thing when the Lee-Kramer shear press is used. However, replacing the standard Lee-Kramer cell with penetrometer needles has resulted in significant correlations at the 1% level with r's of 0.5-0.6 with the longissimus dorsi muscle of pork (Hinnergardt and Tuomy, 1970).

The Armour tenderometer has been used by Carpenter et al. (1972) and Henrickson et al. (1972) on ribbed carcass beef to determine the relationship of raw beef tendermeat and technological taste panel evaluations were -0.84 and -0.78. Simple linear correlation coefficients between water-holding capacity, fat, and moisture vs. the technological taste panel evaluation for tenderness were -0.59, 0.32, and 0.36, respectively. However, results of the multiple correlation analysis using water-holding capacity, protein, moisture, fat, and ash were borderline when coupled with the maximum raw penetration force in improving the relationship of the maximum penetration force of raw meat to the technological taste panel evaluation.

ness to the tenderness of cooked beef. Henrickson et al. (1972) found that the Armour tenderometer readings on raw beef were not highly related to the cooked beef tested with the Warner-Bratzler shear force machine with r's of -0.01 to -0.15. Carpenter et al. (1972) found they could reduce the variability of tenderness groups (tender and nontender) of U. S. Choice from 23 to 9%. When they related the tenderometer readings to a trained panel evaluation of tenderness, the linear correlations were low (-0.15)to -0.35)

The USDA grade is often taken as an indication of tenderness of beef. However, this has not been established firmly in the literature. Simone et al. (1959), Rhodes et al. (1958), and Tuomy et al. (1961) found no significant differences in tenderness attributable to grade. On the other hand, Cover et al. (1958) and Doty and Pierce (1961) found a trend toward increased palatability with higher carcass grades.

The effect of fat present in meat on tenderness has been studied by a number of investigators, with mixed results. Covington et al. (1970), McBee and Wiles (1967), Field et al. (1966), Alsmeyer et al. (1959), and Helser et al. (1930) found significant relationships between tenderness and fat content. On the other hand, many investigators found no significant relationships, including Breidenstein et al. (1968), Howard and Judge (1968), Cover et al. (1956), Suess et al. (1966), and Goll et al. (1965).

It is evident that many of the predictors for cooked meat tenderness reported in the literature have some validity, but none of them is adequate in itself, particularly when compared against taste panel results. The one that

Food Laboratory, U. S. Army Natick Laboratories, Natick, Massachusetts 01760.

comes the closest is the penetrometer, but even though it is very useful, there is considerable room for improvement. There is a possibility that the various predictors are indicating different facets of tenderness and multiple regression techniques could be used to advantage by bringing them together. Therefore, this study was undertaken first to prove out the penetrometer with beef and second to explore the use of multiple regression with several parameters.

#### EXPERIMENTAL METHODS

The study was conducted in two phases, with the procedures being the same for both but with some differences in the data obtained. In phase I, four each of biceps femoris and longissimus dorsi muscles from USDA grade choice beef were studied. In phase II, a total of 48 muscles were studied, with 24 each from USDA choice and good carcasses. The 24 from each grade were further divided into 12 each of longissimus dorsi and 12 each of biceps femoris muscles.

The beef was received as boneless bottom rounds (9-14 kg) and beef ribs (8.6-14.6 kg) at intervals during the study. The meat was received fresh from local commercial establishments. The biceps femoris and longissimus dorsi muscles were dissected out when the meat was received. The meat was then double-wrapped with freezer paper and frozen in a blast freezer at  $-29^\circ$ . The frozen muscles were cut into steaks 1.3-cm thick, 20 steaks per muscle. A circular portion 6.4 cm in diameter was cut from each steak, wrapped in aluminum foil, and stored at  $-29^{\circ}$  until evaluated. The 20 steaks from each muscle were maintained in order and divided into five groups, A through E, anterior to posterior, with four steaks in each group. Within each group the four numbered steaks were treated as follows: steak No. 1, proximate analysis; steak No. 2, tenderness panel evaluation; steak No. 3, penetrometer and panel tenderness evaluation; steak No. 4, penetrometer

A sample for proximate analysis was prepared from each muscle by combining the five no. 1 steaks from each group in a muscle. Analyses were performed in accordance with the Official Methods of Analysis, Association of Official Analytical Chemists (1965). Water-holding capacity (WHC) was performed in accordance with Wierbicki *et al.* (1957).

For penetrometer and taste panel evaluation, steaks no. 2, 3, and 4 were allowed to equilibrate overnight at 4° and weighed. Steaks no. 3 and 4 were penetrated (raw penetrometer reading). Steaks 2, 3, and 4 (wrapped in aluminum foil) were then cooked to an internal temperature of  $82^{\circ}$  in a steam chamber, equilibrated to 4°, and weighed, and no. 3 and 4 were penetrated (cooked penetrometer reading). Steaks 2 and 3 were presented to the technological taste panel for tenderness evaluation.

Penetration of the steaks was accomplished according to the method of Hinnergardt and Tuomy (1970) using an Allo-Kramer Shear Press modified with a five-needle penetrometer head. Readings were expressed in kilograms of force representing the average of three peak values of the time force curve taken at three different locations within each steak. Thus, the entire steak may be sampled for tenderness by penetration without affecting the tenderness evaluation of the technological taste panel.

The technological taste panel consisted of ten members experienced in the evaluation of beef tenderness. The meat was rated on a 9-point scale on which 5 would be considered optimum tenderness, 1 extremely tough, and 9 extremely tender.

#### RESULTS AND DISCUSSION

In both phases of the study, good linear correlations were found between the raw penetrometer readings and

Table I. Simple Linear Correlations. Phase I

×	у	r (59)	F (1-57)
Raw penetrometer	Ash	0.29	5.45%
Raw penetrometer	Fat	$-0.23 \text{ n.s.}^{a}$	3.21 n.s.4
Raw penetrometer	H₂O	0.29%	5.25%
Raw penetrometer	WHC	0.59°	30.62
Raw penetrometer	Taste panel	-0.84°	139.15°
Ash	Taste panel	—0.17 n.s.ª	1.61 n.s.ª
Fat	Taste panel	0.325	6.405
H₂O	Taste panel	0.36°	8.59°
WHC	Taste panel	-0.59°	30.310

 $^{\rm a}$  n.s. = not significant at the 5% level.  $^{\rm b}$  Significant at the 5% level.  $^{\rm c}$  Significant at the 1% level.

Table II. Multiple Correlations. Phase I (N = 48)

	x's in r	R	F
y = Taste panel	1, 2, 3	0.87	54.84°
$\mathbf{x}_1 = raw penetrometer$	1,2	0.85	72.80°
$\mathbf{x}_2 = WHC$	1,3	0.50	72.99°
$x_3 = \%$ fat	2, 3	0.68	26.15°
	3 after 1 and 2		$5.98^{b}$
	2 after 1 and 3		5.27%
	1 after 2 and 3		$58.51^{\circ}$
y = Taste panel	1, 2, 3	0.85	48.19°
$x_1 = raw penetrometer$	1, 2	0.85	72.80°
$\mathbf{x}_2 = WHC$	1,3	0.85	70.93°
x₃ = % ash	2,3	0.64	19.06°
	3 after 1 and 2		0.44 n.s.ª
	2 after 1 and 3		1,48 n.s.ª
	1 after 2 and 3		63.74°
y = Taste panel	1, 2, 3	0.88	51.85°
$x_1 = raw penetrometer$	1, 2	0.88	78.02°
$\mathbf{x}_2 = \%$ protein	1,3	0.88	78.02°
$x_3 = \%$ fat	2,3	0.24	1.43 n.s.ª
	3 after 1 and 2		0.66 n.s.ª
	2 after 1 and 3		0.66 n.s.ª
	1 after 2 and 3		143.62°
y = Cooked			
penetrometer	1, 2, 3	0.96	163.37°
$\mathbf{x}_1 = raw penetrometer$	1, 2	0.96	241.35°
$\mathbf{x}_2 = \%$ protein	1, 3	0.96	243.57°
<b>x</b> ₃ == % fat	2, 3	0.29	2.13 n.s.ª
	3 after 1 and 2		1.55 n.s.ª
	2 after 1 and 3		1.17 n.s.ª
	1 after 2 and 3		443.87°
y  = Taste panel	1, 2, 3	0.898	61.24°
$\mathbf{x}_1 = Cooked$			
penetrometer	1, 2	0.898	93.94°
$x_2 = \%$ protein	1, 3	0.898	93.80°
$x_3 = \%$ fat	2, 3	0.239	1.36 n.s.ª
	3 after 1 and 2		0.00 n.s.ª
	2 after 1 and 3		0.06 n.s.ª
	1 after 2 and 3		$170.71^{\circ}$

 $^{a}$  n.s. = not significant at the 5% level.  $^{b}$  Significant at the 5% level.  $^{c}$  Significant at the 1% level.

the taste panel results with r = -0.84 in phase I and r = -0.78 in phase II (Tables I and III). This indicates that the penetrometer can be very useful, especially in experimental work, where it has the big advantage of being a nondestructive t $\checkmark$ . The correlations were better than those found within the previous work on pork (Hinnergardt and Tuomy, 1970).

Table I shows that there is a significant linear correlation between WHC and the raw penetrometer reading. At the same time, there is a significant linear correlation between the taste panel results on the cooked meat and the WHC with r's of the same order of magnitude. When multiple correlation coefficients including both raw penetrom-

Table III. Simple Linear Correlations. Phase I	Table III.	Simple	Linear	Correlations.	Phase	11
--	------------	--------	--------	---------------	-------	----

x	У	r (48)	<b>F</b> (1-47)
Fat	Raw penetrometer	0.346	6.02 <sup>b</sup>
H <sub>2</sub> O	Raw penetrometer	0.19 n.s."	1.77 n.s."
Fat	Taste panel	—0.16 n.s."	1.28 n.s.ª
H <sub>2</sub> O	Taste panel	0.06 n.s.«	0.20 n.s.ª
Fat	Cooked penetrometer	0.21 n.s."	2.24 n.s."
H <sub>2</sub> O	Cooked penetrometer	—0.07 n.s."	0.25 n.s.«
Fat	Cook loss	0.01 n.s. <sup>a</sup>	0.00 n.s.ª
H <sub>2</sub> O	Cook loss	0.15 n.s."	1.08 n.s."
		r (240)	F (1-239)
Raw penetrometer	Taste panel	-0.78°	374.26
Cooked penetrometer	Taste panel	-0.84°	569,94°
Cook loss	Taste panel	-0.48°	70.94°
Cook loss	Raw penetrometer	0.47"	<b>69.41</b> °
Cook loss	Cooked penetrometer	0.54	<b>99.84</b> °
Raw penetrometer	Cooked penetrometer	0.88	809.48

" n.s. = not significant at the 5% level. Significant at the 5% level. Significant at the 1% level.

Table IV. Multiple Correlations. Phase II (N = 59)

	x's in r	r	F
y = taste panel	1, 2, 3	0.86	52.90°
$x_1 = raw penetrometer$	1, 2	0.85	76.21°
$x_2 = \% H_2 O$	1,3	0.86	79.86°
🛪 = % protein	2,3	0.37	4.58
	3 after 1 and 2		2.42 n.s.ª
	2 after 1 and 3		0.47 n.s.ª
	1 after 2 and 3		128.62°
y  = taste panel	1, 2, 3	0.88	51.71°
$x_1 = raw penetrometer$	1, 2	0.88	74.84°
$x_2 = \% H_2O$	1, 3	0.88	78.69°
$x_3 = \%$ fat	2, 3	0,23	1.23 n.s.ª
	3 after 1 and 2		2.03 n.s.ª
	2 after 1 and 3		0.28 n.s.ª
	1 after 2 and 3 14		144.79°

 $^{\alpha}$  n.s. = not significant at the 5% level.  $^{b}$  Significant at the 5% level.  $^{c}$  Significant at the 1% level.

Table V. Analysis of Variance for Taste Panel Results. Phase II

	Degrees of	% of		
Factor	freedom	Significance	variance	
USDA grade (A)	1	b	0.5	
Muscle (B)	1	с	75.9	
Area in muscle (C)	4	ь	6.2	
AB	1	n.s.ª		
AC	4	n.s.ª		
BC	4	ь	1.0	
Remainder	224		16.4	

 $^a$  n.s. = not significant at 5% level.  $^b$  Significant at 5% level.  $^c$  Significant at 1% level.

eter and WHC (Table II) are obtained against y = taste panel results, there is no improvement in the coefficients obtained using raw penetrometer results alone. In one analysis, the F value for WHC after the other variables are taken out is significant at the 5% level, which would indicate a possibility that the inclusion of WHC may possibly have some use in a regression equation but it should be studied further. It would appear from the good correlation obtained between WHC and the raw penetrometer results that they are at least partially testing the same factor.

The fat content of beef, particularly as fat marbling, is often taken as an indication of tenderness. However, in this investigation, fat was shown to be a rather poor indicator. In Table I a significant correlation at the 5% level is shown between fat and taste panel results. However, the correlation coefficient is only 0.32, which is nowhere near as high as the r obtained when the raw penetrometer values are considered. In Phase II no correlation was found (Table III). Similar results are found when the percent of fat is used as a factor in multiple correlations (Table II and IV). This suggests that the value of fat in multiple regression equations for predicting tenderness is marginal, but further work is certainly indicated before it should be ruled out, particularly with other meats such as pork. Water follows the same pattern as fat. This could be expected since normally there is a direct relationship between fat and H<sub>2</sub>O in raw meat.

In Table I, the ash content of the raw meat and the raw penetrometer reading are shown to correlate at the 5% level. However, with an r of only 0.2954, this correlation is rather poor and in Table II it is shown that ash does not improve the multiple correlation where y = taste panel results.

Analysis of variance results for phase II are shown in Table V. The muscle used (longissimus dorsi or biceps femoris) was significant at the 1% level and accounted for 75.9% of the variance observed. The USDA grade was significant at the 5% level but only accounted for 0.5% of the variance observed, which makes it only a very small part of the differences found by the taste panel. However, only two grades (choice and good) were used in the study and a larger effect might have been found if the lower grades had been included. The areas in the muscle were also significant at the 5% level, but accounted for 6.2% of the variance.

In general, this study has shown excellent correlations between the raw penetrometer readings and the technological taste panel results on the same piece of beef. Thus, the method can be valuable in experimental studies where natural variability in the raw material confronts the experimenter with comparatively large unexplained variations in his results. It has the big advantage of being nondestructive, so that the same piece of meat can be tested more than once at different stages of a study and then submitted to a taste panel. However, the results using various other factors in multicorrelation analysis are not so clear cut. It is believed that more work should be done along these lines to determine if even better predictions of cooked meat tenderness can be obtained.

It should be noted that there are good correlations between the raw penetrometer *vs.* cooked penetrometer and cooked penetrometer *vs.* taste panel (Table II). These relationships can be very useful in experimental work where it is difficult or inconvenient to use a taste panel. This is particularly true with large studies where panel fatigue and costs become important factors.

### LITERATURE CITED

- Association of Official Agricultural Chemists, Official Methods of Analysis, Washington, D. C., 10th ed., 1965.
  Alsmeyer, R. H., Palmer, A. Z., Kroger, M., Kirk, W. G., Proc. 11th Res. Conf. Res. Counc. Amer. Meat Inst. Found. Univ. Chicago (1959)
- Black, W. H., Warner, K. F., Wilson, C. V., USDA Tech. Bull. 217, 43 (1931)
- Zii, 43 (1931).
   Breidenstein, B. B., Cooper, C. C., Cassens, G. E., Bray, R. W., J. Anim. Sci. 27, 1532 (1968).
   Carpenter, Z. L., Kauffman, R. G., Bray, R. W., Weckel, K. G., Food Technol. 19, 1424 (1965).
- Carpenter, Z. L., Smith, G. C., Butler, O. D., J. Food Sci. 37, 126
- (1972). Cover, S., Butler, O. D., Cartwright, T. C., J. Anim. Sci. 15, 464
- (1956). Cover, S., King, G. T., Butler, O. D., Tex. Agr. Exp. Sta. Bull.
- 889 (1958)
- Covington, R. C., Tuma, H. J., Grant, D. L., Dayton, A. D., J. Anim. Sci. 30, 191 (1970). Doty, D. M., Pierce, J. C., USDA Tech. Bull. 1231 (1961).
- Field, R. A., Nelms, G. E., Schoonover, C. O., J. Anim. Sci. 25, 360 (1966).

FLAVONES FROM GRASS SILAGE

- Goll, D. E., Carlin, A. F., Anderson, L. P., Kline, E. A., Walter, M. J., Food Technol. 19, 845 (1965).
- Helser, M. D., Nelson, P. M., Lowe, B., Iowa Agr. Exp. Sta. Bull. 272 (1930).
- Henrickson, R. L., Marsden, J. L., Morrison, R. D., J. Food Sci. 37,857 (1972).

- 37, 857 (1972).
  Hinnergardt, L. C., Tuomy, J. M., J. Food Sci. 35, 312 (1970).
  Howard, R. D., Judge, M. D., J. Food Sci. 33, 456 (1968).
  McBee, J. L., Naumann, H. D., J. Anim. Sci. 18, 1477 (1959).
  McBee, J. L., Wiles, J. H., J. Anim. Sci. 26, 701 (1967).
  Rhodes, V. J., Naumann, H. D., Kiehl, E. R., Brody, D. E., Cook, R., Mo. Agr. Exp. Sta. Res. Bull. 677 (1958).
  Simone, M., Carroll, F., Chichester, C. O., Food Technol. 13, 337 (1959)
- (1959)
- Suess, G. G., Bray, R. W., Lewis, R. W., Brungardt, V. H., J. Anim. Sci. 25, 1203 (1966). Tuomy, J. M., Lechnir, R. J., Miller, T., QMF & CIAF Report
- No. 27-61, 1961.
- Warner, K. R., Proc. Amer. Soc. Anim. Prod. 114 (1928).
- Wierbicki, E., Kunkle, L. E., Deatherage, F. F., Food Technol. 11,69 (1957).

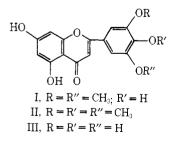
Received for review September 14, 1972. Accepted May 11, 1973. The paper reports research undertaken at the U. S. Army Natick (Mass.) Laboratories and has been assigned No. TP-1246 in the series of papers approved for publication. The findings in this re-port are not to be construed as an official Department of the Army position.

# Structural Determination of Two Basal Metabolic Rate-Stimulating Flavones from **Grass Silage**

David A. Stelzig\* and Syed A. Qasim<sup>1</sup>

Two chemicals from dried grass silage, previously shown to be basal metabolic rate-stimulating when fed to male rats, were shown by ultraviolet spectral analysis and paper chromatography to be tricin and probably 5,7-dihydroxy-3',4',5'-trimethoxyflavone. The basal metabolic rate-stimulating activity of these compounds is greater than certain other flavonoids, probably because they are resistant to degradation by intestinal flora.

McLaren et al. (1964) have shown that dried grass silage (DGS), made from approximately equal parts of wheat, vetch, orchard grass, and alfalfa, stimulated basal metabolic rates (BMR) when fed to male rats. McLaren et al. (1964, 1966) also demonstrated that an 80% ethanol extract of DGS and various other flavonoid-containing extracts were BMR-stimulating to the rat. More recently (Qasim, 1970), DGS was extracted according to the method of McLaren et al. (1964) and subjected to extensive fractionation with solvent extractions and paper chromatography. Several of the fractions, including compounds I and II of this paper, were shown to cause elevated BMR when incorporated into the diet of male rats. The present report offers proof that one of these fractions is the flavone



Division of Plant Sciences, West Virginia University, Morgantown, West Virginia 26506.

tricin (I) and there is strong evidence that the other is 5,7-dihydroxy-3',4',5'-trimethoxyflavone (II).

#### EXPERIMENTAL SECTION

Spectral Analyses. Ultraviolet spectra were measured in 95% ethanol as well as in 95% ethanol saturated with sodium acetate or containing 5% AlCl<sub>3</sub> or 2 N NaOH (Jurd, 1962).

Paper Chromatography. All chromatography was onedimensional on 56-cm sheets of Whatman no. 1 filter paper. The solvents were of analytical reagent quality and were used without further purification except for phenol, which was distilled over zinc dust according to the method of Gage et al. (1951). The solvents employed were 1butanol-acetic acid-water (4:1:5, v/v) (BAW), acetic acid-concentrated HCl-water (30:3:10, v/v) (Forestal solvent), 73% (w/w) phenol in water, 30% (w/v) acetic acid in water and double-distilled water (Seikel, 1962).

Compounds separated by chromatography were routinely located under ultraviolet light after fuming the papers with ammonia. When studying the color properties of the separated chemicals, the chromatograms were sprayed with 5% ethanolic AlCl<sub>3</sub>, 5% aqueous neutral or basic lead acetate, or Folin reagent followed by exposure to ammonia fumes.

Derivatizations. Compound II was demethylated by refluxing in benzene containing AlCl<sub>3</sub> according to the method of Seshadri and Varadarajan (1953). Another sample of compound II was hydrolyzed by refluxing in 2 NHCl for 2 hr, as described by Harborne and Hall (1964).

<sup>&</sup>lt;sup>1</sup> Present address: 179 Luker Road, Luker Gunj, Allahabad V.P. India.