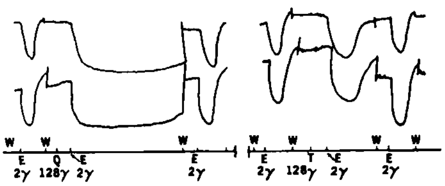

Fig. 1.—Prolongation of epinephrine (E) relaxation time of guinea pig colon segments in the presence of quercetin (Q) and tricin (T). W = wash out and replacement of bath fluid (50 ml.) with fresh Tyrode solution.

bloat symptoms. On the basis of muscle relaxing power, the quercetin administered was equivalent to 100 Gm. of tricin (12).

In another study (13) each of three cows were given 2 Gm. of quercetin on 1 day, followed by 10 Gm. each on 2 successive days. The cows showed no indication of bloat. If we assume a tricin content of 0.02% in alfalfa, it would be impossible for cows to consume enough alfalfa to ingest 10 Gm. of tricin in 1 day.

Mouse Uterine Weight Bioassay Procedure.—In this previously described report (14), weanling mice (five per cage) were fed diets containing the test materials. At the end of 4 days, the mice were sacrificed, and the uteri were excised and weighed. The results are presented in Table I. For comparative purposes, coumestrol was included in the study. The data indicate that tricin was weakly estrogenic ($p = <0.01$).

Metabolic Fate Studies.—That quercetin is absorbed from the intestinal tract is shown by the appearance of three split products in the urine when

TABLE I.—EFFECT OF TRICIN, COUMESTROL, AND DIETHYLSTILBESTROL ON THE IMMATURE MOUSE UTERUS

Group[a]	Compd.	Quantity Fed per Mouse, mg.	Mean Uterine Wt., mg.
1	Control	0	9.6 ± 0.25
2	Tricin	20	11.2 ± 0.57
3	Tricin	40	11.4 ± 0.75[b]
4	Tricin	40	12.4 ± 1.02[b]
5	Coumestrol	0.6	32.4 ± 2.63
6	Diethylstilbestrol	0.0001	29.2 ± 0.40

[a] Five weanling female mice per group. Supplements incorporated into control diet and fed 4 days (10 Gm. diet/mouse). [b] $p = <0.01$.

the compound is fed to rats by mouth (15). However, when tricin was fed under similar conditions, no split products like syringic acid were detected. Thus, it is doubtful that tricin is absorbed from the intestinal tract.

REFERENCES

(1) Harborne, J. B., *Chem. Ind.*, **1958**, 1590.
(2) Anderson, J. A., *Can. J. Res.*, **9**, 80(1933).
(3) Ferguson, W. S., Ashworth, De B., and Terry, R. A., *Nature*, **166**, 116(1950).
(4) Ferguson, W. S., *ibid.*, **161**, 816(1948).
(5) Ferguson, W. S., Ashworth, De B., and Terry, R. A., *ibid.*, **163**, 606(1949).
(6) Ferguson, W. S., *Farming (Norwich)*, **4**, 81(1950).
(7) Curnow, D. H., Robinson, T. J., and Underwood, E. J., *Australian J. Exptl. Biol. Med. Sci.*, **26**, 171(1948).
(8) Bickoff, E. M., *et al.*, *Science*, **126**, 969(1957).
(9) Guggolz, J., Livingston, A. L., and Bickoff, E. M., *J. Agr. Food Chem.*, **9**, 330(1961).
(10) Wenzel, D. G., and Rosenberg, P., THIS JOURNAL, **45**, 367(1956).
(11) Wilson, R. H., and DeEds, F., *J. Pharmacol. Exptl. Therap.*, **95**, 399(1949).
(12) Lindahl, I. L., *et al.*, Technical Bulletin No. 1161, U. S. Department of Agriculture, ARS(1957).
(13) Dunkley, W. L., personal communication.
(14) Bickoff, E. M., *et al.*, *J. Animal Sci.*, **18**, 189(1960).
(15) Booth, A. N., *et al.*, *J. Biol. Chem.*, **223**, 251(1956).

Effects of Gibberellic Acid on the Fixed Oils of Four Plants

By C. DWAYNE OGZEWALLA*

The influence of gibberellic acid on the quality of fixed oils from four plants has been studied. Sesame, castor bean, sunflower, and flax plants were treated with aqueous solutions of gibberellic acid when the plants reached blooming size and at 2-week intervals until harvest. The iodine values and the quantity of unsaponifiable matter varied between samples and between oils from different species of plants. Significant variations from normal were produced in castor and sesame oils. The differences consisted of higher saponification values in both oils and a lower acid value in the latter. A higher saponification value indicating shorter chain fatty acids was the only characteristic common to all oils from treated plants. Some of the changes obtained were from plants that had insignificant or no visible morphological differences.

THE GIBBERELLINS have been known in the United States and Europe for only about 12 years, and they have been available for extensive experimental use for less than a decade (1). The number of research papers involving the gibberellins runs into the thousands; there have been a number of reviews which give detailed information regarding such aspects as the physiology, chemistry, and recommended economic applications (1–5).

Sciuchetti (6), in a recent review, has included the influence of gibberellic acid (GA) on volatile oil, glycoside, and alkaloid producing plants. Only a few of the long list of references deal with changes in the active components, and no references are listed relative to changes in fixed oils. The percentages of certain terpenes in volatile oils have been changed by the use of GA. A slight increase

Received May 16, 1963, from the School of Pharmacy, Southwestern State College, Weatherford, Okla.
Accepted for publication April 1, 1964.
* Fellow of the American Foundation for Pharmaceutical Education, 1957–1959. S. B. Penick Fellow, 1958–1959. Present address: University of Cincinnati, Cincinnati, Ohio.
This study was supported in part by a grant from the Frontiers of Science Foundation of Oklahoma.

TABLE I.—CHARACTERISTICS OF FIXED OILS FROM CONTROL AND GA-TREATED PLANTS

Test	Treatment	Sesame Oil	Castor Oil	Sunflower Oil	Linseed Oil
Iodine value	Control	105	90	96	162
	GA	101	88	97	179
	Change	—	—	+	+
Unsaponifiable matter	Control	0.2	0.1	0.3	0.5
	GA	0.6	0.6	1.4	0.3
	Change	+	+	+	—
Saponification value	Control	191	183	199	188
	GA	197	187	200	191
	Change	+[a]	+[a]	+	+
Acid value	Control	35	0.5	10	1.7
	GA	15	0.3	7	1.3
	Change	—[a]	—	—	—
Ester	Control	156	182	189	186
	GA	182	187	193	190
	Change	+	+	+	+

[a] Statistically significant at 0.05 level.

of menthol in peppermint oil was detected (7); published results of gas chromatograms indicated changes in volatile constituents of this same oil, although the peaks were not identified or their percentages calculated (8). The altered morphological changed in belladonna and stramonium which have been observed have been accompanied by changes in the total alkaloid content and in the distribution of alkaloids through plant parts (9). In these plants both the biosynthesis and transportation mechanisms are undoubtedly involved.

The objectives of this experiment were (*a*) to ascertain whether GA would bring about changes in the quality of fixed oils and to determine the direction of those changes and (*b*) to determine if chemical changes are invariably accompanied by visible morphological deviations. Alterations in quantity of oil produced were recorded but were not intended as a part of the study.

EXPERIMENTAL

Flax (*Linum usitatissimum* L. var. redwood, family *Linaceae*) was chosen as one of the study plants because it reportedly does not respond to low concentrations of exogenous GA (10). Three other oil bearing plants that grow under cultivation in Western Oklahoma were used. These are sesame (*Sesamum indicum* L. var. *blanco*, family *Pedaliaceae*), sunflower (*Helianthus annus* L., family *Compositae*), and castor bean (*Ricinus communis* L., family *Euphorbiaceae*). Seeds were planted in a small irrigated garden in 1961 and 1962 at the appropriate time of spring. The soil of the drug plant garden was not homogeneous, and other environmental factors were not uniform. A randomized block design was chosen using two adjoining castor or sunflower plants as a block—one treated and one control. Both sesame and flax plantings were divided into four approximately equal sized blocks; half of each was treated with GA.

When the plants reached blooming size and the first flowers appeared, treatment was initiated using 10 p.p.m. aqueous solution of GA.[1] It was impossible to prevent a spray from blowing from one treatment to another because of persistent winds. This was reduced to an insignificant level by the use of a laundry sprinkler purchased in a dry goods store. Sunflower, sesame, and flax plants were sprinkled until the solution dripped from the leaves. The leaves of castor plants shed the droplets of solution so readily that another method of treatment was chosen. GA was injected into the pith cavity of the stems with a hypodermic needle and syringe. Approximately 0.5 mg. of GA per plant was used on the sunflower and castor plants and smaller amounts on the others. At the time of treatment the large leaves of the sunflower and sesame plants had reached their maximum number, and few leaves increased in size thereafter. Records were kept of the height of the plants. The number, width, and length of leaves were measured and the shape noted.

[1] Gibberellic acid was supplied as the potassium salt through the courtesy of Merck and Co., Inc., Rahway, N. J.

The fruit of sunflower and the seed of the other plants were harvested, threshed, and cleaned by hand. A quantity of sesame seed and sunflower fruit was lost due to natural causes. However, each block of the experiment was harvested at the most opportune time for the particular plant. Seed from each castor plant and from each block of flax and sesame were collected, extracted, and assayed separately. The fruit from the sunflower treatments was pooled because of heavy losses of seed due to insect and storm damage to the flowerheads.

Oil was obtained from the ground seed or fruit by the use of a Soxhlet extractor using benzene as the solvent. The benzene was evaporated at room temperature. U.S.P. XVI (11) procedures were employed to determine iodine value (Hanus method), saponification value, unsaponifiable matter, and free fatty acids. The acid value was calculated from the free fatty acid test and the ester value was obtained by taking the difference between the saponification value and the acid value.

Analysis of variance tests were run using the randomized block design. Notation was made when the resultant F test indicated that the results were significant at the 0.05 level. The results are indicated in Table I.

RESULTS

Morphological changes resulting from GA application were observed in castor plants and consisted of internode elongation and a reduction in the number of capsules and seeds. The height of the treated plants was 8% greater than the controls; there was a 6% reduction in the number of seeds obtained. A reduction of oil per plant was proportional to the reduction in number of seeds. Significant changes were noted in no other plants. A smaller quantity of fruit from sunflower and of seed from sesame was collected; but due to natural losses of the plant parts, no percentage reduction was calculated. An increase of 5% in flax seed was obtained. Further studies will need to be made to verify this finding and to determine if the number of fruits per plant or the number of seeds in each fruit are increased.

Because the oil of sunflower seed was pooled, no statistically significant differences in the quality of the oils were possible. The iodine value and the saponification value of the sunflower oil were somewhat different from those reported in the literature (12). It is known, however, that the composition of sunflower oil varies according to the locality in which it is grown (13). No significant differences were noted in the linseed oil samples. Significant differences in sesame oil consisted of higher saponification values and lower acid values in oils from plants treated with GA. A higher saponification value was the only significant deviation obtained from castor oils.

Saponification values were consistently higher in the oils from treated plants. The average acid value was consistently lower; however, there was considerable variation between individual castor oil samples and the confidence limits overlapped. The iodine values and the percentages of unsaponifiable matter obtained varied between species of oils and between samples.

DISCUSSION

The reduction in yield in several species of oil bearing plants is not surprising, as a smaller percentage of active constituents has been observed by other workers (4). One author (8) was lead to conclude "that gibberellic acid is of doubtful value in drug plant cultivation." While more studies will need to be made to justify such a broad statement, it does illustrate that hoped-for increases have seldom been obtained. An investigation of proper timing, methods of application, and dosage will need to be conducted.

Morphological changes were either insignificant or absent in some of the treated plants; yet there were changes in the quality of the oils. With more refined methods, slight changes may be detected in the plants used. The choice of economic crops for investigation with GA need not be limited to those that show a striking growth response.

Oils produced from plants treated with GA differed from the controls in their chemical characteristics. The increased saponification values indicated that the oils from treated plants are composed of shorter chain fatty acids. This characteristic was constant in all the species and samples of oils tested. It is more likely to be a general physiological effect of GA than any other. Acid values tended to be lower, an indication that more of the fatty acids were esterified. The resultant higher ester values would indicate that a greater percentage of glyceryl esters occurred in the oils from plants treated with GA. Other characteristics varied between species. The photosynthetic products which build the fixed oils are produced in the leaves some distance from the place where they are stored. A physiologically abnormal catabolism or transportation or both might have been involved in the plants used.

SUMMARY

Slight changes in the chemical constants of oils from GA-treated plants were observed. Significant changes were seen in sesame and castor oils.

Increased saponification values from all treated plants indicate that shorter chain fatty acids were present in the oils. Acid values tended to be lower, an indication of less free fatty acids. Iodine values and unsaponifiable matter varied among species.

Changes were observed in the quality of oils obtained from plants that had insignificant or no visible morphological differences.

REFERENCES

(1) Stodola, F. H., "Source Book on Gibberellin 1828–1957," Agriculture Research Service, U. S. Dept. of Agriculture, Peoria, Ill., 1958.
(2) Brian, P. W., *Biol. Rev.*, 34, 37(1959).
(3) Stowe, B. B., and Yamaki, T., *Ann. Rev. Plant Physiol.*, 8, 181(1957).
(4) Stuart, N. W., and Cathey, H. M., *ibid.*, 12, 369 (1961).
(5) Wittwer, S. H., and Bukovac, M. J., *Econ. Botany*, 12, 213(1958).
(6) Sciuchetti, L. A., This Journal, 50, 981(1961).
(7) Ogzewalla, C. D., *Dissertation Abstr.*, 20, 4085(1960).
(8) Gjerstad, G., *Planta Med.*, 8, 127(1960).
(9) Smith, G. M., and Sciuchetti, L. A., This Journal, 48, 361(1959).
(10) Marth, P. C., Audia, W. V., and Mitchell, J. W., *Botan. Gaz.*, 118, 106(1956).
(11) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 895.
(12) Spector, W. S., "Handbook of Biological Data," W. B. Saunders Co., Philadelphia, Pa., 1956.
(13) Mirov, N. T., *Lloydia*, 26, 117(1963).

ERRATA

In the review article titled "Absorption of Implanted Solid Drug" (1), the following corrections should be made:

1. Equation 17 contains the term $(D^0 - kt)$. This term should be replaced by $(D^0r - kt)$.
2. Equation 23 contains the term $2K^2/K^3$. This should be replaced by $2k^2/K^3$.
3. Algebraic manipulation alone will reduce Eq. 29 to 29a, and the discussion in the paragraph following these equations should be interpreted in light of this fact.

(1) Ballard, B. E., and Nelson, E., This Journal, 51, 915 (1962).