this was fed to an alkaloid-free leaf. As with nicotine-1′-oxide we were unable to detect any conversion to nornicotine. Consequently, it is doubtful that during the conversion the methyl carbon passes through the oxidation level of formic acid while still attached to the pyrrolidine nitrogen atom.

The hydroxymethyl derivative, with its carbon at the oxidation level of formaldehyde, has not been tested yet. Other attractive possibilities, such as quaternary *N*-derivatives are also being considered as intermediates.

Summary

Tobacco scions were grafted onto tomato stocks to provide tobacco leaves essentially free of pyridyl alkaloids. These leaves were used for testing the intermediary role of nicotine-1′-oxide in the conversion of nicotine to nornicotine.

Nicotine-1′-oxide was neither converted to nornicotine, nor did it inhibit the conversion of nicotine. These findings eliminate nicotine-1′-oxide from consideration as an intermediate.

Preliminary evidence indicating that *N*′-formyl nornicotine does not play a significant role in the conversion has also been obtained.

Acknowledgment

The authors gratefully acknowledge the technical assistance of Mr. John Whipple and of Mr. Walter H. Bass.

Literature Cited

1. Dawson, R. F. 1942. Accumulation of nicotine in reciprocal grafts of tomato and tobacco. Am J. Botany 29: 66–71.
2. Dawson, R. F. 1951. Alkaloid biogenesis. III. Specificity of the nicotine-nornicotine conversion. J. Am. Chem. Soc. 73: 4218–21.
3. Dawson, R. F. 1960. Biosynthesis of the Nicotiana alkaloids. Am. Scientist 48: 321–40.
4. Egri, L. 1957. Untersuchung über die Alkaloide einiger Tabaksorten mit besonderer Berücksichtigung der Erforschung der Biosynthese von Nornikotin. Fachliche Mitt. Oesterr. Tabakregie 19–24.
5. Rayburn, C. H., W. R. Harlan, and H. R. Hanmer. 1950. Rearrangement of nicotine oxide. J. Am. Chem. Soc. 72: 1721–23.
6. Stephens, R. L. and J. A. Weybrew. 1959. Isatin: a color reagent for nornicotine. Tobacco Sci. 3: 48–51.
7. Stepka, W. and L. J. Dewey. 1961. Conversion of nicotine to nornicotine in harvested tobacco: fate of the methyl group. Plant Physiol. 36: 592–97.
8. Wenkert, E. 1954. Oxidation in the biogenesis of alkaloids. Experientia 10: 346–50.

Physiological Effects of Gibberellic Acid. VI. Other Gibberellins in Three Test Systems [1]

L. Paleg, D. Aspinall, B. Coombe, and P. Nicholls [2]

Department of Plant Physiology, Waite Agricultural Research Institute, Adelaide, Australia

Introduction

The results of recent investigations have indicated that the known gibberellins (4) vary widely in their effects on various species. GA_4, GA_7, and GA_9 for example, are extremely active on cucurbits (4, 9), while only GA_7 exerts a strongly promotive effect on the flowering of Silene (11). Conversely, GA_9, although active on some species, shows no activity on Meteor dwarf peas or the germination of lettuce seed both of which respond well to GA_2, GA_3, and GA_4.

The effects of 7 gibberellins and allogibberic acid on 5 responses of 3 test systems are presented here, as well as a discussion of the biological necessity for a lactone ring in gibberellin-like compounds.

[1] Received Aug. 1, 1963.

[2] Present address: Division of Biology, California Institute of Technology, Pasadena, California.

Material and Methods

Samples of the various gibberellins and allogibberic acid were generously supplied by Drs. Grove and Brian of Imperial Chemical Industries, Ltd. The compounds were dissolved by shaking in doubly glass distilled water. Under these conditions insufficient GA_7 went into solution to test. Experiments were carried out within 2 weeks of preparing the solutions and the same solutions were used in all of the tests described below.

Lettuce Radicle and Hypocotyl Elongation. The conditions employed were similar to those described by Frankland and Wareing (7). Nine-cm petri dishes containing about 200 lettuce seeds (var. Grand Rapids), 2 filter paper discs and 5 ml water each, were placed under fluorescent light (1500 f-c) at 20° for 48 hours. Germinating seeds were selected

for uniformity and placed in 9-cm petri dishes (12/dish) with 1 filter paper disc and 6 ml of test solution. Lengths of radicles and hypocotyls were measured after 4 days and each value presented is the average of 3 replicates.

Sugar and Nitrogen Release from Endosperm. Estimations were made as outlined previously (13, 14). Eight endosperm (cut from previously sterilized barley seed, var. Triple Awned Lemma) were placed in 5-cm petri dishes (8 per dish) and incubated in 3 ml of test solution, including 500 μg streptomycin sulfate, at 30° for 22 hours. One ml of the ambient solution within each petri dish was diluted to 10 ml with water, shaken with amberlite IR-120 (H^+) resin, filtered, and assayed for reducing sugar content (17); 1 ml was also taken for protein N determinations, in which a Lowry (10) reaction was employed.

Enlargement of Grapes. Currant grape berries (4.0 $\pm$ 0.5 mm in diameter) on 6 vigorous vines were treated 1 month after flowering. Each vine was 1 replicate carrying 23 treatments. Treatments were restricted to individual clusters (23/vine), and applied to one branch, of 15 to 40 berries, on each cluster by brushing about 0.1 ml solution onto the berry surfaces. No wetting agents were used and, as a result, large droplets formed on the berries. The berries were harvested at maturity, about 10 weeks after treatment, and the 10 largest berries on each twig were weighed.

Results

During the initial testing of the stimulation of lettuce hypocotyl elongation with GA_3, it was noticed that the radicle occasionally appeared to respond to treatment. Because of this the radicle responses to the varous gibberellins were determined. Tables I and II present the lengths of hypocotyls and radicles, respectively, after 4 days in the test solutions.

All compounds stimulated hypocotyl growth significantly, except GA_8, which was inactive. The order of activity is similar to, though not identical with, that obtained by Brian et al. (4): GA_3 and GA_4 are most active whereas GA_6 is least active; GA_1, GA_5, and GA_9 have intermediate activity. Allogibberic acid is about as active as GA_6.

Table I

Effect of Gibberellins on Lettuce Hypocotyl Extension (length in cm)

Significant differences ($P > 0.05$): between means in body of table, 0.85; between GA means, 0.50; between concentration means, 0.30.

Substance	Concentration (mg/l)			
	0.1	1.0	10.0	Mean
H_2O				2.3
GA_1	3.7	5.3	7.6	5.5
GA_3	3.9	6.1	7.8	5.9
GA_4	4.6	7.3	7.1	6.3
GA_5	2.9	3.9	6.7	4.5
GA_6	3.0	3.4	5.0	3.8
GA_8	2.3	2.3	2.3	2.3
GA_9	3.6	4.8	6.3	4.9
Allogibberic	3.3	3.5	4.9	3.9
Mean	3.4	4.6	6.0	

Table II

Effect of Gibberellins on Lettuce Radicle Extension (length in cm)

Least Significant differences ($P > 0.05$): between means in body of table, 2.54; between GA means, 1.47; between concentration means, 0.90.

Substance	Concentration (mg/l)			
	0.1	1.0	10.0	Mean
H_2O				7.7
GA_1	7.2	8.8	10.3	8.8
GA_3	9.1	9.0	10.5	9.5
GA_4	8.0	9.9	9.4	9.1
GA_5	7.9	8.2	11.2	9.1
GA_6	10.8	11.6	10.1	10.8
GA_8	8.2	7.6	9.5	8.4
GA_9	9.9	8.8	10.8	9.8
Allogibberic	9.6	10.2	12.8	10.9
Mean	8.8	9.3	10.6	

Interestingly, the order of activity is not the same for radicle elongation as for hypocotyl elongation. Judging by the means, allogibberic acid and GA_6 are most active, GA_8 is inactive, and the other gibberellins are intermediate. The values for radicles are not as uniform as those for hypocotyls, but particularly at the lowest concentration, GA_6 exerted a far stronger effect than the others.

Figure 1 is a plot of hypocotyl lengths and radicle lengths at each concentration. Analysis indicated a highly significant ($p > 0.001$) correlation between these 2 indices suggesting that an increase in concentration of an active gibberellin will stimulate both hypocotyl and radicle elongation, rather than 1 organ at the expense of the other. Although there were no significant differences between the slopes for the individual gibberellins, highly significant differences between the means of the regressions were obtained indicating, again, that some of the gibberellins seem to stimulate 1 organ more effectively than the others.

Responses produced by the various gibberellins in the barley endosperm reaction are illustrated in figures 2 and 3. The similarity between sugar release and protein release is evident. In these 2 endosperm tests, the gibberellins can be divided into 3 groups. GA_1 and GA_3 seem to initiate the 2 responses at the lowest concentrations, with GA_4 only slightly less active. Allogibberic acid, GA_5, and GA_6 have low levels of activity, while GA_8 and GA_9 are considered to be inactive since a 1 part in 10,000 contamination of these compounds, with either GA_1 or GA_3, could cause similar results.

Figure 4 presents the values obtained with grapes as the test system. Here, again, GA_1 and GA_3 are

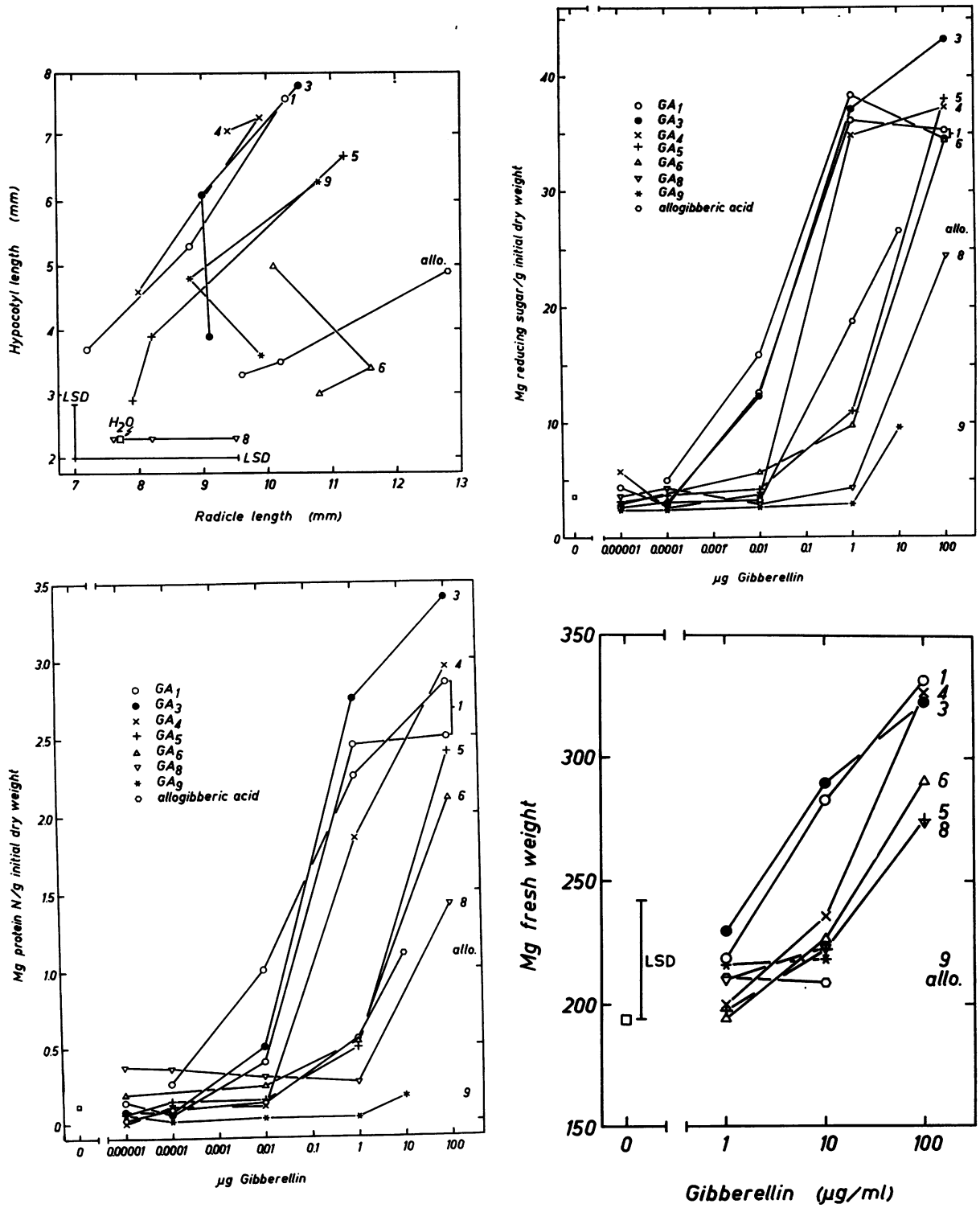

FIG. 1 (*upper left*). The effect of various gibberellins (as numbered) at 3 concentrations on the lengths of the radicle and hypocotyl of lettuce seedlings. LSD ($P > 0.05$) is shown for each axis.

FIG. 2 (*upper right*). Reducing sugar release by barley endosperm induced by different concentrations of various giberellins.

FIG. 3 (*lower left*). Protein-nitrogen release by barley endosperm induced by different concentrations of various gibberellins.

FIG. 4 (*lower right*). The effect of 3 concentrations of various gibberellins on the fresh weight of grape berries.

most active in that they cause the greatest berry enlargement at the lowest concentration. GA_4, GA_5, GA_6, and GA_8 may be grouped together, whereas, due to the lack of sufficiently high concentrations, it was not possible to determine whether GA_9 and allogibberic acid are active at all in this test.

Crude measurements of total sugar content of the berries were made with an Abbé refractometer. None of the test values differed significantly from each other, although they were all lower than the control. The inverse correlation between sugar content and berry weight was, however, significant.

Discussion

The results obtained with lettuce seedlings indicate that gibberellins are capable of stimulating both hypocotyl and radicle elongation. Usually, gibberellins are described as having little promoting effect on the root growth of intact plants (3). Note, however, that almost all of the experiments on which this description is based, were carried out with GA_3, and the possibility exists that, as in this work, other gibberellins can stimulate radicle or root growth more effectively than GA_3. Alternatively, if the gibberellins were enhancing processes concerned with the mobilization of reserves in the endosperm or cotyledons, a stimulation (albeit indirect) of hypocotyl and/or radicle elongation might be observed. At least 2 other reports of GA_3-stimulated radicle growth have appeared (5, 15) which may be similarly explained. In both of these, the stimulatory effect of treatment eventually diminished, as might be expected if an endogenous supply of reserves became exhausted. This type of effect on storage reserves has been described (14) for cereals and is further supported by the results reported here with barley.

The close relationship between sugar and protein nitrogen release from barley endosperm was demonstrated earlier (14). In this work, the similarity between the 2 responses induced by the 8 gibberellins is further evidence for the conclusion that both responses are controlled by the same initial gibberellin effect. The fact that some gibberellins are more active than others, and that some are even inactive, suggests that the initial gibberellin reaction is reasonably specific.

The gibberellin-induced enlargement of grapes is well documented and previous tests of GA_1, GA_2, GA_3, and GA_4 indicated that GA_2 was less active than the 3 other gibberellins (18). This work has confirmed the previous tests in so far as GA_1 and GA_3 are more active than any of the other gibberellins tested.

The apparent specificity of various gibberellins in different test systems has been brought out in particular by the responses of cucurbits to GA_4, GA_7, and GA_9 (1). Four of the five measurements of gibberellin activity reported here have not indicated any gross differences in effectiveness from that usually reported, in that GA_1, GA_3, and GA_4 are most active, while GA_8 is either least active or inactive. The activity of the gibberellins in the fifth response, lettuce radicle elongation, does not conform to the other results. In this test, GA_6 and allogibberic acid exhibit considerably greater effects than the other gibberellins. The reason for this divergence is not understood.

The biological activity of allogibberic acid has been the subject of much debate. Early work by Yabuta et al. (19) suggested that allogibberic acid (then called gibberellin B) had some biological activity in that it stimulated the growth of rice seedlings. Brian et al. (2) tested allogibberic acid on cress seedlings and found, rather than a stimulation, an inhibition of root growth at the highest concentrations used. Other workers (6, 8) have also concluded that allogibberic acid has no biological activity.

The only recent work suggesting that allogibberic acid may be biologically active, is that of Murfet and Barber (12) who demonstrated that allogibberic acid, like GA_3, could delay flowering, but, unlike GA_3, had no effect on stem elongation. The fact that it was active in one system but inactive in the other, while GA_3 was active in both, made it unlikely that the positive response was caused by contamination with GA_3.

That the lactone structure is unnecessary for biological activity also receives support from the recent finding of Ruddat, Lang, and Mosettig (16). Steviol, a plant terpenoid (with a 6-membered B ring), also lacks a lactone structure and yet produces a gibberellin-like elongation response in dwarf (mutant d-5) corn seedlings.

Allogibberic acid is derived by mild acid hydrolysis of GA_3, and may be found in aged solutions of GA_3 kept at room temperature (2). In view of the apparent activity of allogibberic acid in the tests described in this work, the possibility must be considered that some of the responses that have been described as being due to GA_3 may, in fact, be due to one of its degradation products, allogibberic acid.

Summary

Seven gibberellins and allogibberic acid were tested for their ability to cause 5 responses in 3 test systems. No novel activities were observed for any of the gibberellins although allogibberic acid demonstrated biological effectiveness in 2 of the test systems. It appears likely that a reevaluation of the biological necessity for the lactone structure in gibberellin-like compounds is required.

Literature Cited

1. Brian, P. W. 1961. Response of the cucumber hypocotyl to 2 new gibberellins. Nature, 189: 74.
2. Brian, P. W., J. F. Grove, H. G. Hemming, T. P. C. Mulholland, and M. Radley. 1958. Gibberellic acid. Part VI. The biological activity of allogibberic acid and its identity with gibberellin B. Plant Physiol. 33: 329–33.
3. Brian, P. W., J. F. Grove, and J. MacMillan. 1960. The gibberellins. Progr. Chem. Org. Natl. Prods. 18: 350–433.

4. BRIAN, P. W., H. C. HEMMING, AND D. LOWE. 1962. Relative activity of the gibberellins. Nature, 193: 946–8.
5. BROWN, C. AND E. M. GIFFORD, JR. 1958. The relation of the cotyledons to root development of pine embryos grown in vitro. Plant Physiol. 33: 57–64.
6. BUKOVAC, M. J. AND S. H. WITTWER, 1961. Biological evaluation of gibberellins A_1, A_2, A_3 and A_4 and some of their derivatives. In: Plant Growth Regulation. R. M. Klein, ed. Iowa State Press, Ames. p 505–20.
7. FRANKLAND, B. AND P. F. WAREING. 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature, 185: 255.
8. HALEVY, A. H. AND H. M. CATHEY. 1960. Effects of structure and concentration of gibberellins on the growth of cucumber seedlings. Botan. Gaz. 122: 63–7.
9. LOCKHART, J. A. AND P. H. DEAL. 1960. Prevention of red light inhibition of stem growth in the Cucurbitaceae by gibberellin A_4. Naturwissenschaften 47: 141–2.
10. LOWRY, O., N. ROSEBROUGH, A. FARR, AND R. LAUDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–75.
11. MICHNIEWICZ, M. AND A. LANG. 1962. Effect of 9 different gibberellins on stem elongation and flower formation in cold-requiring and photoperiodic plants grown under noninductive conditions. Planta 58: 549–63.
12. MURFET, I. AND N. BARBER. 1961. Effect of gibberellic acid and allogibberic acid on flowering and internode length in *Pisum sativum*. Nature 191: 514–5.
13. PALEG, L. 1961. Physiological effects of gibberellic acid. III. Observations on its mode of action on barley endosperm. Plant Physiol. 36: 829–37.
14. PALEG, L., B. G. COOMBE, AND M. S. BUTTROSE. 1962. Physiological effects of gibberellic acid. V. Endosperm responses of barley, wheat, and oats. Plant Physiol. 37: 798–803.
15. RICHARDSON, S. D. 1958. Radicle elongation of *Psudotsuga menziesii* in relation to light and gibberellic acid. Nature 181: 429–30.
16. RUDDAT, M., A. LANG, AND E. MOSETTIG. 1963. Gibberellin acitivity of steviol, a plant terpenoid. Naturwissenschaften 50: 23.
17. SOMOGYI, M. 1952. Notes on sugar determination. J. Biol. Chem. 195: 19–23.
18. WEAVER, R. J. 1961. Growth of grapes in relation to gibberellin. Adv. in Chem. 28: 89–108.
19. YABUTA, T., Y. SUMIKI, K. ASO, T. TAMURA, H. IGARASHI, AND K. TAMARI. 1941. Biochemistry of the bakanae fungus. X. The chemical constitution of gibberellin (I). J. Agric. Chem. Soc. Japan 117: 721–30.

Metabolism of Threo-D_S-Isocitric Acid in Detached Leaves of Bryophyllum calycinum [1]

Attila O. Klein [2]

Biochemical Laboratory, The Connecticut Agricultural Experiment Station, New Haven, Connecticut

Introduction

Diurnal fluctuations in the organic acid content of leaves is a widely studied property of Crassulacean plants (6). In the dark large amounts of malic acid accumulate, presumably in the vacuole, while in the light such stores are depleted (2, 5). The behavior of citric acid shows similar fluctuations but of a smaller amplitude (5, 14). Isocitric acid, the major organic acid component of some species, shows erratic behavior. In *Bryophyllum calycinum,* isocitrate remains remarkably constant throughout a 24-hour period of alternating light and darkness (5, 10, 11), but in *B. daigremontianum* and *Kalanchoe crenata,* isocitrate occasionally varies more than citrate. The presence of a large and constant pool of a component of the citric acid cycle during sharp fluctuations in the levels of 2 other members of the cycle prompted the following question: Does isocitric acid participate in the large scale organic acid transformations of Crassulacean metabolism? The availability in this laboratory of *threo*-D_SL_S-isocitric acid-3,4-C^{14}, of which the D_S-isomer is identical with the natural acid (5) suggested the feeding experiments reported in this paper[3]. The distribution of label among the major acids of *B. calycinum* leaves when fed specifically labeled D_S-isocitric acid indicates that this compound does indeed supply carbon atoms to malic and other acids in the course of a diurnal cycle.

[1] Aug. 2, 1963.

[2] Present address: Department of Biology, Brandeis University, Waltham 54, Massachusetts.

[3] The nomenclature of isocitric acid used in this paper is that recently advocated by Vickery (16).