Phytochemistry, 1964, Vol. 3, pp. 93 to 101. Pergamon Press Ltd. Printed in England

PLANT HORMONES IV. IDENTIFICATION OF THE GIBBERELLINS OF *ECHINOCYSTIS MACROCARPA* GREENE BY THIN LAYER CHROMATOGRAPHY

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(*Received* 7 *June* 1963)

Abstract—Using thin layer chromatography in conjunction with bioassay and spectrofluorimetry, gibberellic acid and gibberellins A_1, A_4 and A_7 have been identified in an acidic extract of the seed of *Echinocystis macrocarpa* Greene. In addition, this extract has yielded compounds (I) and (II) and a new gibberellin of unknown structure which appears to be related to gibberellin A_7.

INTRODUCTION

IN PART III[1] the identification of gibberellic acid in immature barley and grass by thin layer chromatography was described. The present paper records the application of this technique to the identification of microgram amounts of gibberellic acid and gibberellins A_1, A_4 and A_7 in an acidic extract of the seed of *Echinocystis macrocarpa* Greene. In addition, this extract has been found to contain the $1 \rightarrow 3$ lactones (I) and (II) and a new gibberellin, of unknown structure, which appears to be structurally related to gibberellin A_7. Gibberellic acid[1] and gibberellin A_1[2-4] are known plant constituents but gibberellins A_4 and A_7 have hitherto only been identified in culture filtrates of the fungus *Gibberella fujikuroi*.[5-7]

Thin layer chromatography of the gibberellins has been studied by Kutáček *et al.*,[8] Sembdner *et al.*[9] and by MacMillan and Suter.[10] The latter authors have shown that all the nine known gibberellins and their methyl esters, except those of gibberellins A_4 and A_7, can be separated by a combination of adsorption and partition chromatography on thin layers of silica and kieselguhr with di-isopropyl ether–acetic acid, and benzene–acetic acid–water. Recently a two-dimensional development in ethyl acetate followed by water has been shown[1] to give a partial resolution of the known gibberellins on silica and to be a useful system for the purification of the more polar gibberellins such as gibberellic acid.

Several new solvent systems have been examined in the course of the present work and the results are recorded in Table 1. Isopropanol–ammonium hydroxide and n-butanol–

[1] D. F. JONES, J. MACMILLAN and M. RADLEY, *Phytochemistry*, **2**, 307 (1963)
[2] J. MACMILLAN, J. C. SEATON and P. J. SUTER, *Tetrahedron* **11**, 60 (1960).
[3] C. A. WEST and B. O. PHINNEY, *J. Am. Chem. Soc.* **81**, 2424 (1959).
[4] A. KARAWADA and Y. SUMIKI, *Bull. Agr. Chem. Soc. Japan* **23**, 343 (1959).
[5] N. TAKAHASHI, H. KITAMURA, A. KAWARADA, Y. SETA, M. TAKAI, T. TAMURA and T. SUMIKI, *Bull. Agr. Chem. Soc. Japan* **19**, 267 (1955); N. TAKAHASHI, T. SETA, H. KITAMURA, A. KAWARADA and Y. SUMIKI, *ibid.* **21**, 75 (1957).
[6] N. TAKAHASHI, T. SETA, H. KITAMURA and Y. SUMIKI, *Bull. Agr. Chem. Soc. Japan* **21**, 396 (1957); **23**, 405 (1959).
[7] B. E. CROSS, R. H. B. GALT and J. R. HANSON, *Tetrahedron* **18**, 451 (1962).
[8] M. KUTÁČEK, J. ROSMUS and Z. DEYL, *Biol. Planta.* **4**, 226 (1962).
[9] G. SEMBDNER, R. GOSS and K. SCHREIBER, *Experientia*, **18**, 584 (1962).
[10] J. MACMILLAN and P. J. SUTER, *Nature*, **197**, 790 (1963).

ammonium hydroxide are useful for distinguishing gibberellic acid and gibberellin A_7 from their respective isomeric 1→3 lactones (I)[11] and (II)[12] which are readily produced by mild hydrolysis.[12,13] Further differentiation between (II) (R_f 0·75) and gibberellin A_7 (R_f 0·84) and between the methyl ester of (I) (R_f 0·16) and methyl gibberellate (R_f 0·20) can be made on

TABLE 1. THIN LAYER CHROMATOGRAPHIC PROPERTIES OF THE GIBBERELLINS

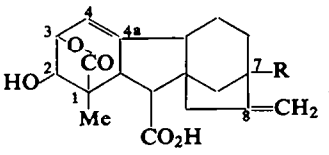
Compound	R_{GA}* (reproducibility in brackets) Solvent systems 1 (±0·02)	2 (±0·03)	3 (±0·05)	4 (±0·03)	Colour of induced fluorescence
Gibberellin A_1	1	1	1	1	Blue
A_2	0·92	0·91	1·1	0·90	Purple
A_3	1	1	1	1	Green–blue
A_4	1·17	1·13	1·48	0	Purple
A_5	1·0	1·12	1·35	0·80	Blue
A_6	1	1·07	0·95	0·95	Blue
A_7	1·17	1·13	1·48	0	Yellow
A_8	0·89	0·84	0·52	1·05	Blue
A_9	1·19	1·25	1·55	0	Purple
Gibberellenic acid[14]	0·50	0·42	0·10	1·03	Green–blue
(I)	1·0	0·92	0·70	1·0	Green–blue
(II)	1·17	1·04	1·36	0	Yellow

Solvent systems: 1, Isopropanol–water (4:1); 2, isopropanol–4·5 N ammonium hydroxide (3:1); 3, n-butanol–4·5 N-ammonium hydroxide (3:1); 4, phosphate buffer (0·1 M, pH 6·3) on silica impregnated with capryl alcohol.

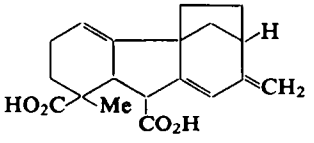
* R_{GA} = movement relative to gibberellic acid (A_3).

Approximate distances travelled by gibberellic acid on development to 10 cm were as follows: 5·5 cm in systems 1, 2 and 5 and 7·0 cm in system 4.

silica with benzene–acetic acid–water. The latter system also separates methyl gibberellenate[14] (R_f 0·84) from methyl gibberellate. Gibberellenic acid,[14] which is formed by mild acid hydrolysis of gibberellic acid, can be separated from the latter by chromatography on silica in water, in isopropanol–water, in isopropanol–ammonium hydroxide, in n-butanol–ammonium hydroxide and in di-isopropyl ether–acetic acid.



(I) R = OH
(II) R = H



(III)

Detection of the gibberellins and their simple derivatives can be effected by spraying with ethanolic sulphuric acid and heating at 120° which renders the compounds visible as fluorescent spots in ultraviolet light.[10] Some distinction between the gibberellins can be made

[11] B. E. CROSS, J. F. GROVE and A. MORRISON, *J. Chem. Soc.* 2498 (1961).
[12] B. E. CROSS and J. R. HANSON, personal communication.
[13] I. KUHR, *Folia Microbiol.* **8**, 358 (1963).
[14] J. S. MOFFATT, *J. Chem. Soc.* 3045 (1960).

on the basis of the colour and induction period of the fluorescence.[1] A precise measurement of the former is afforded by eluting the fluorescent spots from the chromatogram and determining the fluorescence activation and emission spectra in ethanolic sulphuric acid solution. The activation and emission maxima of the known gibberellins, in 50% ethanolic sulphuric acid, are recorded in Table 2. Gibberellic acid and gibberellins A_2, A_4, A_7 and A_9 fluoresce after heating for 25 min at 50°; gibberellins A_1, A_5, A_6 and A_8 do not yield a fluorophor under these conditions, but after heating for 25 min at 100° they give spectra, which are similar to but relatively less intense than, that of gibberellic acid. Gibberellenic acid and compound (I) both give spectra identical to that of gibberellic acid when reacted under the same conditions. Compound (II) similarly resembles gibberellin A_7.

TABLE 2. ACTIVATION AND EMISSION MAXIMA OF THE GIBBERELLINS

Gibberellin	Reaction conditions*	Activation max. (mμ)	Emission	Relative intensity
A_1	1	417	463	~10
A_2	2	390	420	13
		440	468	7
A_3	2	417	463	100
A_4	2	390	420	18
		440	468	8·5
A_5	1	417	463	27
A_6	1	417	463	10–16
A_7	2	390	420	13
		455	473	60
A_8	1	417	463	10–16
A_9	2	390	420	14
		440	468	7

* 25 min in 50% ethanolic sulphuric acid at (1) 100° and (2) 50°.

Gibberellins A_4 and A_7 are distinguished by a comparatively high growth-promoting activity on cucumber[15–17] and this specificity has led to the suggestion[18] that either one or both of these gibberellins may occur naturally in *Curcurbitacea*. The presence of gibberellin-like substances in the wild cucumber *E. macrocarpa* Greene has been demonstrated by West and Phinney[19] and confirmed by Corcoran[20] and Murakami.[21] The nature of these substances has been investigated by West and Reilly[22] who found that an acidic extract was resolved by paper chromatography into two components active on maize mutants. The authors concluded that there were insufficient data on which to base identifications but suggested that the chromatographic and fluorescence properties of one of the components most closely resembled

[15] M. J. BUKOVAC and S. H. WITTWER, *IVth Intern. Conf. Plant Growth Regulation*, Iowa State Univ. Press (1959).
[16] J. A. LOCKHART and P. H. DEAL, *Naturwissenschaften*, **47**, (6), 141 (1960).
[17] P. W. BRIAN, H. G. HEMMING and D. LOWE, *Nature*, **193**, 946 (1962).
[18] J. MACMILLAN, J. C. SEATON and P. J. SUTER, *Advances in Chem. Ser.* **28**, 18 (1961).
[19] C. A. WEST and B. O. PHINNEY, *Plant Physiol* (Supp. 31), XX, (1956).
[20] M. R. CORCORAN and B. O. PHINNEY, *Physiol. Planta*, **15**, 252 (1962).
[21] Y. MURAKAMI, *Botany Mag. Tokyo* **72**, 438 (1959).
[22] C. A. WEST and T. REILLY, *Advances in Chem. Ser.* **28**, 37 (1961).

those of gibberellin A_7 and further, that the other component was similar to, but possibly not identical with, gibberellic acid.

In the present work, the seed (3·5 kg) of *E. macrocarpa** was processed to give a crude acidic extract which showed low but significant activity on the cucumber hypocotyl bioassay.[23] This active material was resolved by chromatography on a column of charcoal–celite in acetone–water mixtures into two groups of active substances; one group being eluted with 45–55% acetone and the other being eluted with 70–75% acetone. The latter group, after partial purification by elution from a column of celite–silicic acid with 10–15% ethyl acetate in chloroform, was resolved by thin layer chromatography on silica in benzene–acetic acid–water into three components *a* (8 μg), *b* (20 μg) and *c* (10 μg).

Components *a* and *b* were identified as gibberellins A_4 and A_7 respectively by the following criteria: (1) the appropriate relative activities[17] on the cucumber hypocotyl test,[23] the lettuce hypocotyl test[24] and the dwarf pea bioassay;[25] (2) the requisite fluorescence spectra; (3) the appropriate R_f values on thin layers of silica in benzene–acetic acid–water,[10] in di-isopropyl ether–acetic acid[10] and in ethyl acetate–water,[1] in each case detection by sulphuric acid (purple and yellow fluorescence respectively) was confirmed by detection by cucumber bioassay; (4—applying to substance *b* only) the requisite R_f values on silica in isopropanol–ammonium hydroxide and in n-butanol–ammonium hydroxide; (5) the appropriate R_f values of the methyl esters, prepared with diazomethane, and of the acetyl derivatives of these esters, prepared by heating in acetic anhydride, on silica in di-isopropyl ether–acetic acid.[10] Further confirmation of the identity of substance *a* was provided by mild acid treatment which gave a product chromatographically identical to gibberellin A_2 on silica in di-isopropyl ether–acetic acid. The latter compound is a known[26] product of the acid treatment of gibberellin A_4.

Component *c* was identified as the isomer (II), of gibberellin A_7 by the following evidence. The component, like (II), showed activity on the cucumber hypocotyl test and gave a fluorescence spectrum identical to that of gibberellin A_7. Substance *c* was chromatographically identical to (II) on silica in benzene–acetic acid–water, in isopropanol–ammonium hydroxide and in n-butanol–ammonium hydroxide. Like (II) and its methyl ester, substance *c* and its methyl ester were chromatographically indistinguishable from gibberellin A_7 and its methyl ester respectively on silica in di-isopropyl ether–acetic acid.

The active material eluted from the charcoal column by 45–55% acetone was resolved by a combination of column chromatography on celite–silicic acid in ethyl acetate–chloroform mixtures and thin layer chromatography on silica in the ethyl acetate–water system[1] into three components *d* (5 μg), *e* (10 μg) and *f* (50 μg).

Component *d* was identified as a mixture of gibberellic acid and gibberellin A_1 by the following findings. On thin layer adsorption chromatography on silica in the ethyl acetate–water system and in di-isopropyl ether–acetic acid the material behaved like gibberellic acid and gibberellin A_1 which are not separated by these systems. However, on partition chromatography on kieselguhr in benzene–acetic acid–water the material was resolved into two components which were identical in mobility, and sulphuric acid-induced fluorescence to

* This material, obtained from S. California through the kindness of Dr. S. Bampton of the Colonial Products Institute, appeared to be fairly mature which would account for the relatively low gibberellin content since Corcoran has shown[20] that the largest amount of extractable gibberellin is present in the seed 27 days after flower wilt.

[23] P. W. BRIAN and H. G. HEMMING, *Nature* **189**, 74 (1961).
[24] B. FRANKLAND and P. F. WAREING, *Nature* **185**, 255 (1960).
[25] P. W. BRIAN and H. G. HEMMING, *Physiol. Planta.* **8**, 669 (1955).
[26] J. F. GROVE, *J. Chem. Soc.* 3545 (1961).

gibberellic acid and gibberellin A_1 respectively. Methylation by ethereal diazomethane gave a product which was resolved on silica in benzene–acetic acid–water into two components identical to methyl gibberellate and gibberellin A_1 methyl ester respectively. Acetylation of the methylation product gave a substance which was resolved by chromatography on silica in di-isopropyl ether–acetic acid into two components identical to di-O-acetyl-methyl gibberellate and di-O-acetyl-gibberellin A_1 methyl ester respectively. The gibberellic acid-like component was revealed by sulphuric acid treatment and brief heating as a green-blue spot and the gibberellin A_1-like component was revealed as a bright blue spot after prolonged heating.

Although the components were not separately bioassayed the mixture of gibberellic acid and gibberellin A_1 showed activity on the lettuce hypocotyl test. This mixture also showed a fluorescence spectrum identical to that of gibberellic acid and produced under similar conditions.

Component *e* was identified as the isomer (I) of gibberellic acid as follows. The substance showed a fluorescence spectrum identical to that of gibberellic acid and produced under similar conditions but, like (I), was inactive on the pea bioassay. The substance was chromatographically identical to (I) on thin layers of silica in n-butanol–ammonium hydroxide and gave a methyl ester identical to the methyl ester of (I) on silica in benzene–acetic acid–water. Like (I), component *e* was chromatographically indistinguishable from gibberellic acid on silica in the ethyl acetate–water system, and in di-isopropyl ether–acetic acid and also on kieselguhr in benzene–acetic acid–water. Vigorous acid hydrolysis gave a product which was chromatographically identical to gibberic acid[28] on silica in di-isopropyl ether–acetic acid. Gibberic acid is a known[11] acid hydrolysis product of (I).

The lactones (I) and (II) may be present in the plant but in view of their ease of formation it seems likely that they are derived from gibberellic acid and gibberellin A_7 respectively during the isolation procedure.

Component *f* appears to be a new gibberellin; eluted from a celite–silicic acid column in part by ethyl acetate but mainly by acetone it showed activity in the cucumber, lettuce and pea bioassays. Isolation of pure material was accomplished by thin layer chromatography on silica in the ethyl acetate–water system; recovery was effected by extraction of the adsorbent with aqueous sodium bicarbonate followed by acidification and extraction with ethyl acetate. This recovery procedure was necessary since the substance could not be extracted from the silica by acetone, ethanol or water. Subsequently it was found that gibberellins A_1 and A_8 were likewise difficult to extract.

The methyl derivative of substance *f*, prepared by diazomethane methylation of the crude active fraction, was readily freed from the other plant esters by thin layer chromatography on silica in dilute aqueous acetic acid followed by recovery in ethanol. This purified methyl derivative was chromatographically identical to the methylation product of the purified substance *f*. Further, since the pure substance *f* showed the same R_f values in several solvent systems as the substance in the crude active fraction, it was concluded that no modification had occurred during the rather drastic recovery procedure.

Substance *f* showed growth-promoting activity in the cucumber, lettuce and pea bioassays but the small amount of material available precluded a quantitative study. The thin layer and paper chromatographic properties of substance *f* and its methylation product, together with those of gibberellin A_8, the most polar of the known gibberellins, are recorded in Table 3.

[27] Y. MURAKAMI, *Botany Mag. Tokyo* **72**, 36 (1959).
[28] J. MACMILLAN, J. C. SEATON and P. J. SUTER, *Tetrahedron*, **18**, 349 (1962).

Detection was effected by sulphuric acid in the usual way, which rendered component *f* visible as a bright yellow spot closely similar in appearance and induction period to that given by gibberellin A_7, and confirmed on paper chromatograms by bio-assay on cucumber.

Substance *f* shows an interesting anomaly in its chromatographic behaviour. Thus, in predominantly organic solvent systems it is appreciably less mobile than any of the known gibberellins but in a predominantly aqueous development medium the substance has a mobility similar to that of gibberellic acid and less than that of gibberellin A_8. The methylation product shows a similar behaviour. Analogous properties are shown by gibberellin A_2

TABLE 3. CHROMATOGRAPHIC PROPERTIES OF SUBSTANCE *f* FROM *E. macrocarpa*

Solvent system	Adsorbent	R_{GA} (±0·02)	
		Substance *f*	Gibberellin A_8
Free acids			
1	S	0·97	1·08
2	S	0·35	0·85
3	S	0·55	0·87
3	P	0·81†	0·95
4*	S	0·70	0·84
5*	S	0·50	0·55
6*	K	origin†	0·15
7	S	0·6†	0·62
8	C	1·08†	1·20
Methylation products	R_f value (±0·02) relative to methyl gibberellate		
3	S	0·89	1·00
5*	S	origin†	0·13
9	S	0·25	0·70
10	S	1·02	1·09

S = silica gel; K = kieselguhr gel; P = Whatman No. 1 filter paper; C = silica gel impregnated with capryl alcohol.

Solvent systems: 1, water; 2, di-isopropyl ether–acetic acid (1:1); 3, isopropanol–water (4:1); 4, isopropanol–4·5 N-ammonium hydroxide (3:1); 5, n-butanol–4·5 N ammonium hydroxide (3:1); 6, benzene–acetic acid–water (8:3:5); 7, isopropanol–water–ammonium hydroxide (d, 0·88) (10:1:1); 8, phosphate buffer (0·1 M, pH 6·3) saturated with capryl alcohol; 9, di-isopropyl ether–acetic acid (2:1); 10, water–acetic acid (95:5).

* Partition chromatography.

† Single determination.

and its ester which are less mobile than gibberellic acid and methyl gibberellate respectively in *both* di-isopropyl ether–acetic acid and water.

Although, in general, substance *f* appears to be more polar than any of the known gibberellins it is appreciably less so than the gibberellin-like substances reported by Murakami[27] to be present in the immature seeds of several leguminous plants.

The close similarity in sulphuric acid induced fluorescence between compound *f* and gibberellin A_7, referred to above, is reflected in the fluorescence spectrum which is reproduced in Fig. 1. This spectrum, like that of gibberellin A_7, shows an activation peak at 455 mμ and an emission peak at 475 mμ. The spectrum differs from that of gibberellin A_7 in showing a weak secondary activation peak at approximately 427 mμ. It seems unlikely that this

additional peak is due to an impurity since it was present in the same relative intensity in the spectra of several samples and also in the spectrum of the methylation product which was identical to that shown in the figure. The rate at which gibberellin A_7 and substance *f* formed their respective fluorophors in 50% ethanolic sulphuric acid at 20° was found to be the same.

The growth-promoting activity of substance *f* in conjunction with its spectroscopic similarity to gibberellin A_7 suggests a gibbane[29] carbon skeleton. Assuming such a nucleus some further structural features may be tentatively defined as follows. An exocyclic methylene group at position 8 seems likely since gibberellin-like compounds having this grouping reduced do not fluoresce in sulphuric acid solution; gibberellin-like compounds having an oxygen atom in place of the methylene group are biologically inactive[30] and give fluorescence

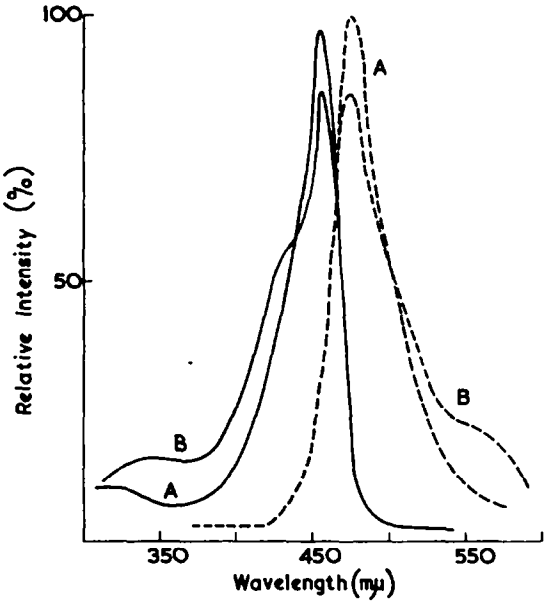


FIG. 1. FLUORESCENCE ACTIVATION AND EMISSION SPECTRUM IN 50% ETHANOLIC SULPHURIC ACID OF SUBSTANCE *f* FROM *E. macrocarpa*.

Key: full line = fluorescence activation; broken line = fluorescence emission.
A = gibberellin A_7 (1 μg); B = substance *f*.

spectra similar to that of gibberellic acid. The close similarity in fluorescence induction period between substance *f* and gibberellin A_7 makes it unlikely that the former contains an hydrated exocyclic methylene group similar to that present in gibberellin A_2[26] since the latter shows a longer fluorescence induction period than does the corresponding non-hydrated gibberellin A_4.[7]

The nature of the species responsible for the sulphuric acid induced fluorescence of gibberellin A_7 has been defined[31] by Speake as a protonated form of (III). The formation of a similar species by compound *f* implies at the least a similar level of oxidation. Some support for this view and an indication of the possible presence of a gibb-3-ene 1 → 4a lactone system

[29] J. F. GROVE and T. P. C. MULHOLLAND, *J. Chem. Soc.* 3007 (1960).
[30] T. P. C. MULHOLLAND, personal communication.
[31] R. N. SPEAKE, *J. Chem. Soc.* 7, (1963).

was provided by the following finding. Thin layer chromatography of substance *f* in n-butanol-ammonium hydroxide, after prolonged equilibration, revealed a small amount of a new substance showing similar fluorescence to substance *f* but having a slightly lower R_f value. On the same chromatogram, gibberellic acid was similarly partially converted into a slower moving compound having chromatographic and fluorescent properties identical to those of (I). The latter transformation, which has also been observed[30] to accompany paper partition chromatography of gibberellic acid in ammoniacal solvents, is due to partial isomerization of the gibb-3-ene 1→4a lactone system to the gibb-4-ene 1→3 lactone system and it seems possible that the partial transformation of compound *f* may be due to a similar isomerization.

The spectroscopic resemblance to gibberellin A_7 suggests either that a 7-hydroxyl group is absent or, less likely, that it is present but protected in such a way as to inhibit the Wagner–Meerwein rearrangement which is a necessary stage[31] in the formation of the fluorophor from gibberellic acid and from the other known 7-hydroxyl containing gibberellins.

EXPERIMENTAL

Thin layer chromatography, detection by sulphuric acid and recovery of material from thin layer chromatograms was accomplished as described in previous publications.[1,10] Development in the solvent system listed in Table 1 was carried out to 10 cm. Equilibration prior to partition chromatography was carried out for 16 hr. Impregnation of silica gel G with capryl alcohol was accomplished by developing the chromoplate in a 7% solution of capryl alcohol in light petroleum (40–60°) and drying at room temperature (1 hr). All chromatographic identifications were made by direct comparison with authentic specimens. The R_f values of the gibberellins not quoted in the present paper have been recorded in previous publications.[1,10]

Bioassays were carried out on the following systems: cucumber hypocotyl,[23] lettuce hypocotyl[24] and dwarf pea.[25]

Methylations were accomplished in methanolic solution by treatment with a slight excess of ethereal diazomethane and acetylation of methyl esters was carried out by heating in acetic anhydride (1 hr) followed by evaporation under reduced pressure. Acid hydrolysis of *Substance a* was accomplished by standing in 3N-hydrochloric acid at room temperature for 2 days. Acid hydrolysis of *Substance c* was carried out by heating in 3N-hydrochloric acid at 100° (2 hr). Detection of gibberic acid was accomplished by spraying with 0·5% aqueous potassium permanganate spray which rendered the compound visible as a yellow spot.[1]

Isolation of the Gibberellins

The seed (3·5 kg) of *Echinocystis macrocarpa* Greene was extracted by the published procedure[28] to give a crude acidic extract (1·7 g) which showed activity on the cucumber bioassay. This acidic extract was chromatographed on a column of charcoal–celite in acetone–water mixtures as described by MacMillan *et al.*[2] and each of the fractions thereby obtained was adsorbed onto silicic acid (2 g) by evaporation of an acetone solution, and placed on top of a column (18 × 1·5 cm) of celite (10 g): silicic acid (5 g). Elution was carried out in 50-ml fractions with chloroform containing increasing proportions of ethyl acetate (5 steps) and finally in acetone. The gibberellin-like substances in the fractions from the latter columns were separated and purified by thin layer chromatography. The results obtained are summarized in Table 4. Estimates of the amounts of pure gibberellins were made by fluorimetry.

TABLE 4. SEPARATION OF GIBBERELLINS FROM *Echinocystis macrocarpa*

Substance, μg	Identity	Charcoal column % acetone	Silicic acid column % ethyl acetate	Thin layer system for separation
a, 8	Gibberellin A_4			Silica with benzene–acetic acid–water[10] (8:3:5)
b, 20	Gibberellin A_7	70–75	15–20	
c, 10	II			
d, 5	Gibberellin A_1+A_3	55	50	Two-dimensional system of ethyl acetate and water on silica[1]
e, 10	I	50	40	
f, 50	Unknown	45–50	100% & acetone	

* Estimated as gibberellin A_7 equivalent.

Spectrofluorimetry

Spectrofluorimetric analyses were made by means of an Aminco-Bowman Spectrophotofluorometer using an I.P.21 photomultiplier. The values and spectra presented are uncorrected for photomultiplier response and source emission. The temperature and acid concentration necessary for maximum fluorophor formation was not the same for all gibberellins. In the work described, except where otherwise stated, the following standard conditions were adopted. Samples were dissolved in 50% ethanolic sulphuric acid and these solutions were then heated at 50° for 25 min or at 100° for the same period.

Gibberellin spots on thin layer chromatograms, located with the aid of marker spots, were eluted with ethanol and the eluate reacted as described above. Alternatively, the chromatograms were sprayed with ethanolic sulphuric acid, heated and the fluorescent spots eluted with ethyl alcohol. The eluants were evaporated down to dryness, the residue dissolved in 50% ethanolic sulphuric acid and the spectrum was determined immediately and again after a period of heating. Background values were obtained by using similar eluates from blank chromatograms.

Acknowledgements—We thank Miss M. Radley for the bio-assays, Messrs. D. Brown and D. Harding for technical assistance and Dr. B. E. Cross for the gift of specimens.