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ISOLATION, IDENTIFICATION AND BIOLOGICAL PROPERTIES OF GIBBERELLIN A_{14}* FROM *GIBBERELLA FUJIKUROI*

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Abstract—A crystalline gibberellin has been isolated from the ethyl acetate-extractable acidic fraction of culture filtrates of *Fusarium moniliforme* Sheld. The compound appears to be gibberellin A_{14}, based on chromatographic mobilities and spectral properties. Chemical and physical evidence for the structure of the molecule is consistent with the structure already proposed for gibberellin A_{14}. The compound is active in the *dwarf*-1, *dwarf*-2 and *dwarf*-5 maize assays and in the cucumber seedling assay for gibberellin-like substances. It is inactive, or only slightly active, in the barley endosperm assay when incubated for 48 hr, although preliminary results indicate the compound is active with longer incubations.

INTRODUCTION

AN EXAMINATION of extracts of culture filtrates of *Fusarium moniliforme* Sheld (imperfect stage of *Gibberella fujikuroi*) revealed an apparently new gibberellin in addition to several of the gibberellins known to be produced by this fungus.[1] This new substance could be induced to fluoresce on thin layer chromatographic plates after exposure to sulfuric acid and heat, and also showed biological properties characteristic of gibberellins, but its chromatographic properties differed from any of the then-known gibberellins. The new gibberellin was purified from this source and structural studies were undertaken. These investigations led to the tentative conclusion, reported earlier,[2] that this was a C_{20} gibbane derivative of molecular formula $C_{20}H_{28}O_6$ substituted with two tertiary methyl, an exocyclic methylene, two carboxyl and two alcoholic hydroxyl groups. While this work was in progress a preliminary report by Cross, Galt and Hanson which described the isolation and characterization of several C_{20} gibberellins was published.[3] Complete accounts of the structural studies and proposed

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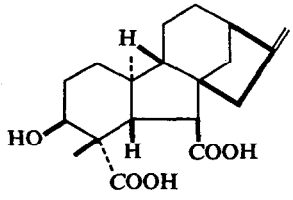
[1] L. G. PALEG, *Ann. Rev. Plant Physiol.* **16**, 291 (1965).

[2] KENNETH C. JONES, Doctoral Dissertation, University of California, Los Angeles (1965) and a talk presented at the meeting of the Pacific Division of the American Association for the Advancement of Science, June (1965).

[3] B. E. CROSS, R. H. B. GALT and J. R. HANSON, *Regulateurs Naturels de la Croissance Vegetale* 265, Centre Natl. Recherche Sci., Paris, (1964).

structures of these C_{20} gibberellins, designated gibberellins A_{12}, A_{13}, A_{14} and A_{15} have appeared more recently.[4–7]

A comparison of properties reported for gibberellin A_{14}, for which structure I was reported by Cross,[6] and the gibberellin isolated in the present work suggested many similarities. An exchange of samples confirmed these similarities, especially in chromatographic properties, but differences were noted in the i.r. spectra of the two samples. Further investigations and comparisons of additional properties now lead us to propose that the gibberellin we isolated is identical with gibberellin A_{14}. This paper will describe our results in the isolation of this gibberellin, studies of its chemical properties, the basis for its identification as gibberellin A_{14}, and some of its biological properties.



RESULTS

Ethyl acetate-extractable acidic components were obtained from 35 l. of culture filtrate of *F. moniliforme* grown as described in the Experimental Section. Gibberellin A_{14} was purified by successive column chromatographic separations on (1) Celite 1 M phosphate buffer, pH 6·2, developed with light petroleum-ethyl ether solvent mixtures, (2) silicic acid developed with chloroform-ethyl acetate solvent mixtures, and (3) Celite-charcoal developed with methanol. A white solid obtained from the third column was crystallized from an ethyl acetate-petroleum ether mixture. Recrystallization yielded 68 mg of needle-like crystals. Thin layer chromatography of 10 μg portions of the recrystallized sample on Silica gel in the isopropyl ether-acetic acid and the benzene-acetic acid-water solvent systems described by MacMillan and Suter[8] revealed a single fluorescing component at R_f values of 0·50 and 0·67, respectively.

The crystalline gibberellin decomposed at 242–243° (uncorrected) after it was extensively dried over P_2O_5 in a vacuum at 80°. Microanalysis of samples dried in this way showed C, 66·1 per cent; H, 7·8 per cent; and O (by difference) 26·0 per cent. (Theory for $C_{20}H_{28}O_5$ is C, 69·0 per cent; H, 8·0 per cent; and O 23·0 per cent. Theory for $C_{20}H_{28}O_6$ is C, 65·9 per cent; H, 7·7 per cent; and O, 26·4 per cent.) Osmometric molecular weight measurements gave a value of 356 ± 18. High resolution mass spectrometry showed a molecular ion at m/e 348·1934 corresponding to a molecular formula of $C_{20}H_{28}O_5$.

The compound showed only end-absorption in the u.v. region with a maximum at approximately 202 nm ($\epsilon = 11{,}000$ M^{-1} cm^{-1}) characteristic of the exocyclic methylene group found in gibberellins.[9] The i.r. absorption spectrum is shown in Fig. 1. Some of the absorption maxima and assignments are: 3450 cm^{-1} (aliphatic hydroxyl), 2960 cm^{-1} (carboxyl hydroxyl),

[4] B. E. CROSS and K. NORTON, *J. Chem. Soc.* 1570 (1965).
[5] R. H. B. GALT, *J. Chem. Soc.* 3143 (1965).
[6] B. E. CROSS, *J. Chem. Soc.* C, 501 (1966).
[7] J. R. HANSON, *Tetrahedron* **23**, 733 (1967).
[8] J. MACMILLAN and P. J. SUTER, *Nature* **198**, 790 (1963).
[9] J. F. GROVE, *J. Chem. Soc.* 3541 (1961).

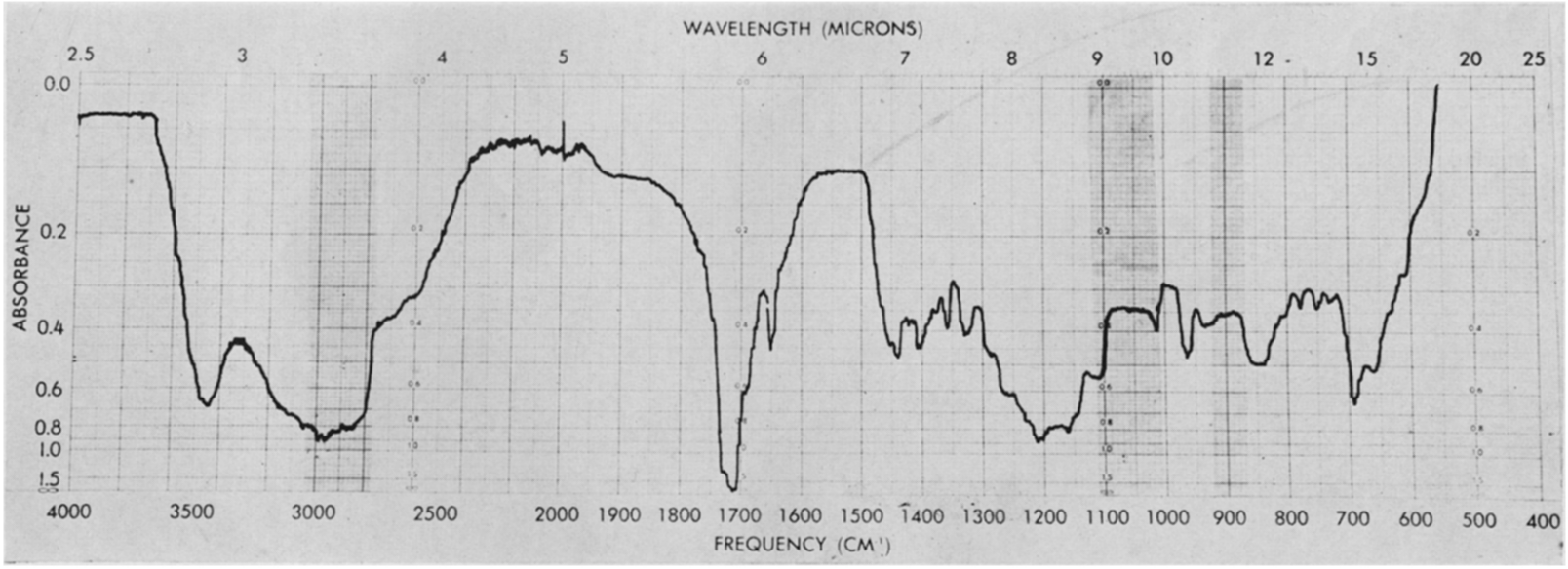


FIG. 1. INFRARED SPECTRUM OF GIBBERELLIN A_{14} MEASURED IN TETRAHYDROFURAN SOLUTION.

[facing page 284]

1710 cm^{-1} (carboxyl carbonyl), and 1650 cm^{-1} and 858 cm^{-1} (exocyclic methylene). The i.r. absorption spectrum measured in a KBr pellet was very similar. Some of the characteristics of the proton magnetic resonance spectrum of the potassium salt measured in D_2O are listed in Table 1.

A spectrophotometric estimation of the degree of unsaturation of the compound by reaction with neutral permanganate indicated the presence of 0·99 non-conjugated ethylenic double bond per molecule, assuming a molecular weight of 348.

TABLE 1. PROTON MAGNETIC RESONANCE DATA

Sample	Peak position	Relative number of protons	Structural assignment
Potassium salt in D_2O	9·29	3	$-\overset{\vert}{\underset{\vert}{C}}-CH_3$
	8·96	3	$-\overset{\vert}{\underset{\vert}{C}}-CH_3$
	6·43	1	$H-\overset{\vert}{\underset{\vert}{C}}-COO^-$
	6·05	1	$H-\overset{\vert}{\underset{\vert}{C}}-O-$
	5·18	2	$\diagdown C=CH_2$
Methyl ester in $CDCl_3$	9·30 (sharp)	3	$-\overset{\vert}{\underset{\vert}{C}}-CH_3$
	8·82 (sharp)	3	$-\overset{\vert}{\underset{\vert}{C}}-CH_3$
	7·63 (doublet; J=12 c/s)	1	10 a proton
	6·63 (doublet; J=12 c/s)	1	$H-\overset{\vert}{\underset{\vert}{C}}-COO^-$
	6·50 (sharp)	1	$-O-H$
	6·32 (sharp)	6	$-O-CH_3$
	5·83 (broad)	1	$H-\overset{\vert}{\underset{\vert}{C}}-O-$
	5·13 (broad)	2	$\diagdown C=CH_2$

Potentiometric titration of aqueous solutions of the isolated compound yielded a neutralization equivalent of 188 with a pK_a of about 4·5 estimated from the shape of the titration curve. Treatment of a sample of the acid with diazomethane yielded a methyl ester which migrated 1·2 times as far as the acid on a silica gel plate developed with the isopropyl ether-acetic acid solvent system.[8] The methyl ester could not be crystallized even after purification on a silicic acid column.

Mass spectral analysis of the methyl ester revealed a molecular ion at m/e 376 corresponding to a molecular formula of $C_{22}H_{32}O_5$ (equivalent to a dimethyl ester of $C_{20}H_{28}O_5$). Some of the characteristics of the proton magnetic resonance spectrum of the ester measured in $CDCl_3$ are summarized in Table 1.

The presence of an alcoholic hydroxyl group was also inferred from treatment of the methyl ester with acetic anhydride. A single acetylated derivative which migrated 1·4 times as far as the methyl ester was detected by its fluorescence on a silica gel thin layer chromatogram developed with the isopropyl ether-acetic acid solvent system.[8]

Biological activity was determined in the *dwarf*-1, *dwarf*-2 and *dwarf*-5 maize assays,[10] the cucumber seedling assay,[11] and the barley endosperm assay.[12] The results of the maize and cucumber assays are shown in Fig. 2. In all maize assays, the log of the response is proportional to the log of the dosage, to a dosage of 10 μg per plant. In most cases, the response to 100 μg of the compound was equal to, or less than, the response to 10 μg. At dosages of 10 μg or less, the compound showed 10 per cent or less of the activity of GA_3 in the *dwarf*-1 and *dwarf*-2 assays, but approximately 100 per cent of the activity of GA_3 in the *dwarf*-5 assay. At dosages of 100 μg of the compound per plant, the activity is approximately 1 per cent that of GA_3 in the three assays.

TABLE 2. BIOLOGICAL ACTIVITY OF THE SAMPLE AND OF GA_3 IN A BARLEY ENDOSPERM ASSAY. EACH VALUE IS THE GEOMETRIC MEAN OF THREE DETERMINATIONS

GA_3		Sample	
Dosage (g)	Absorbancy	Dosage (g)	Absorbancy
1×10^{-6}	1·81	1×10^{-6}	0·29
1×10^{-7}	1·52	1×10^{-7}	0·00
1×10^{-8}	0·65	1×10^{-8}	0·00
1×10^{-9}	0·01	1×10^{-9}	0·00
1×10^{-10}	0·00	1×10^{-10}	0·00

In the cucumber assay, the response to 100 μg of the compound was approximately equal to the response to 10 μg. At dosages of 10 μg per plant or less, the compound was approximately 10 per cent as active as GA_3, while at 100 μg dosages, it was only 1 per cent as active as GA_3.

The results of the barley endosperm assay are shown in Table 2. The compound is inactive in this system at the dosages tested and with an incubation period of 48 hr. However, recent investigations have revealed that the compound is active after longer incubations. Time course studies have determined that this gibberellin causes sugar release to begin approximately 18 hr later than does GA_3. A detailed investigation of the time course of the response to this and other gibberellins is being carried out and will be described elsewhere.

[10] P. M. NEELY, Doctoral Dissertation, University of California, Los Angeles (1959).
[11] M. KATSUMI, B. O. PHINNEY and W. K. PURVES, *Physiol. Plantarum* **18**, 462 (1965).
[12] L. PALEG, D. ASPINALL, B. COOMBE and P. NICHOLLS, *Plant Physiol.* **39**, 286 (1964).

FIG. 2. BIOLOGICAL ACTIVITY OF GIBBERELLIN A_{14} AND GIBBERELLIC ACID IN THE (a) *dwarf*-1, (b) *dwarf*-2, AND (c) *dwarf*-5 MAIZE ASSAYS AND (d) THE CUCUMBER SEEDLING ASSAY.

The vertical bars represent ± one standard deviation of the mean for a treated group of seedlings; the horizontal lines show the mean value, ± one standard deviation of the mean, of the control group. The squares (■) in all cases represent the results with the new gibberellin at the indicated dosages and the circles (●) the results with gibberellic acid at the indicated dosages.

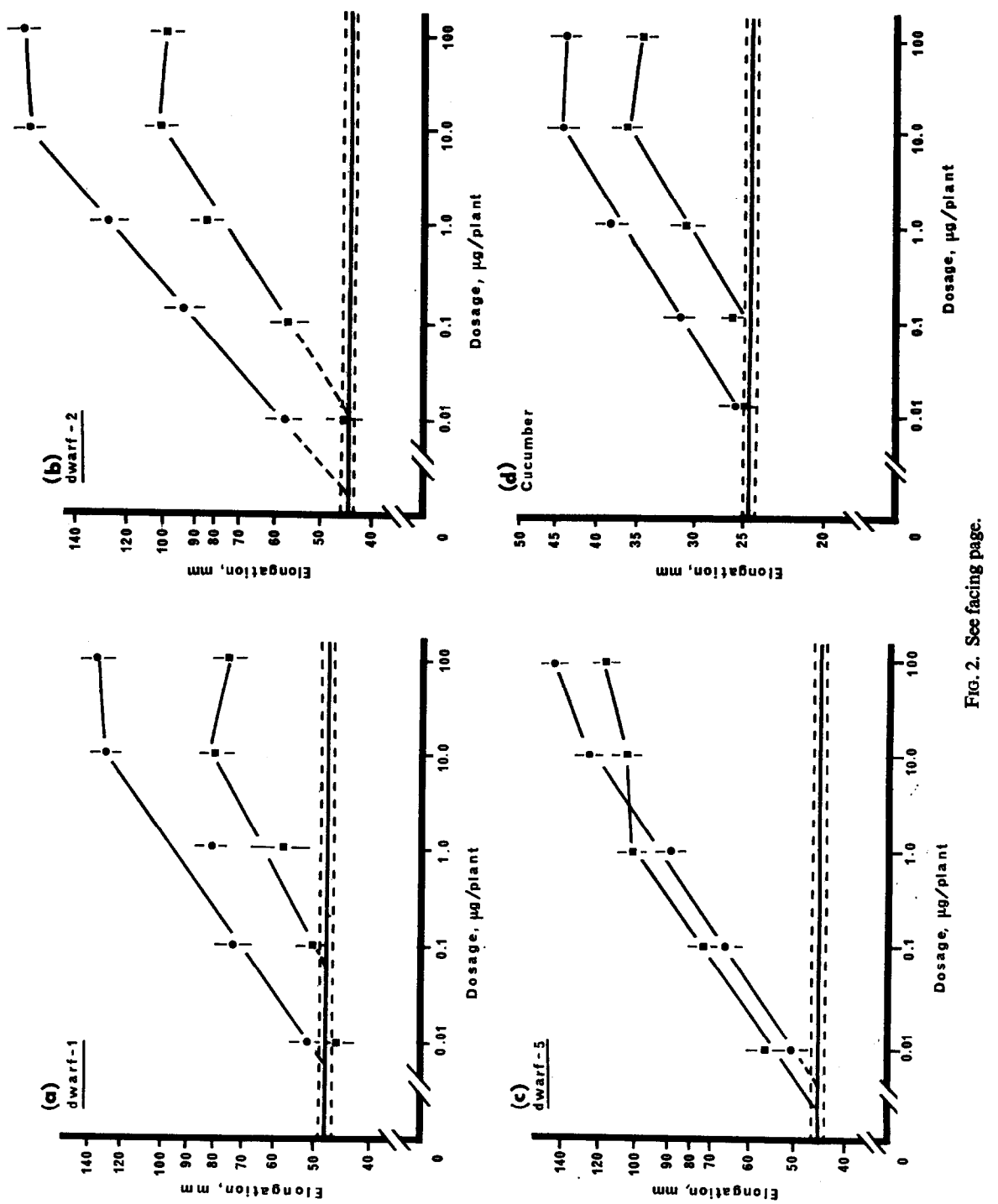


FIG. 2. See facing page.

DISCUSSION

The results presented above are consistent with the presence of two tertiary methyls, an alcoholic hydroxyl, two carboxylic acid groups and an exocyclic methylene in a molecule of formula $C_{20}H_{28}O_5$. The microanalytical data are in better accord with $C_{20}H_{28-30}O_6$, but the mass spectra of both the free acid and the methyl ester leave no doubt that $C_{20}H_{28}O_5$ is the correct formula for the acid. The basis for this discrepancy is not known, although it is possible that the samples subjected to microanalysis were hydrated in spite of the extensive drying procedures. It is further assumed that the functional groups listed above are substituted on the gibbane nucleus common to the gibberellins since this compound showed biological activity in the maize and cucumber assays; these assays are relatively specific for gibbane derivatives and the closely related (−)-kaurene derivatives.[13,14]

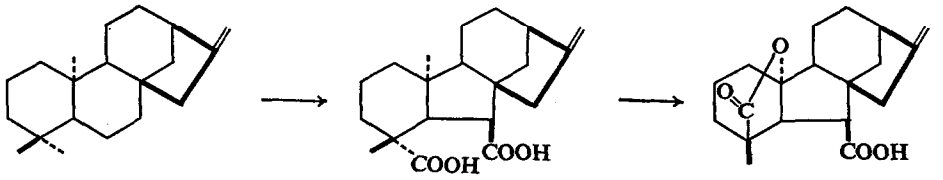
The properties of this gibberellin were obviously similar to those reported by Cross[3,6] for gibberellin A_{14} while this work was in progress. A direct comparison of properties made possible by an exchange of samples with Dr. Cross strongly supports the conclusion that the gibberellin isolated in the present work is indeed identical with gibberellin A_{14}. The two samples co-chromatograph on silica gel plates developed with the isopropyl ether-acetic acid and benzene-acetic acid-water solvent systems. The proton magnetic resonance spectra of the methyl esters of two compounds are virtually identical except for the position of a single peak which is assigned to the hydroxyl proton and, thus, would be expected to vary as a function of the concentration of the sample in the solution for measurement. In addition, comparison of the two compounds by means of gas-liquid chromatography was undertaken by MacMillan using techniques developed in his laboratory.[15] The methyl esters of samples of both compounds showed identical retention times of 4·1 min on a 2 per cent QF-1 column at 201° with a N_2 flow rate of 60 ml/min, and of 22·2 min on a 2 per cent SE-33 column at 190° with a flow rate of 80 ml/min; the trimethylsilyl ethers of the methyl esters also showed identical retention times of 4·9 min and 23·3 min on the same two columns run at 179° and 85 ml N_2/min and 187° and 75 ml N_2/min, respectively. Even more conclusively, MacMillan succeeded in demonstrating that the mass spectra of the derivatives of the two samples obtained from the effluent of the gas-liquid chromatograms were identical.[16]

These properties make it seem certain that the two compounds are the same even though a comparison of the i.r. spectra of the two samples obtained in Nujol muls (by Cross) or in KBr pellets (by the authors) showed distinct differences. However, these differences in spectra obtained from samples in the solid state may be due simply to differences in crystal form. Cross has obtained gibberellin A_{14} in two different crystal forms, and it is possible that the sample isolated in our laboratory was obtained in still a third form or perhaps in a hydrated form. Polymorphism seems to be common among the gibberellins. It should also be noted that differences were also observed in the solution (tetrahydrofuran) i.r. spectrum of a sample of gibberellin A_{14} supplied by Cross from that shown in Fig. 1. This observation was made on a relatively small sample which gave a poorly resolved spectrum. Unfortunately, insufficient gibberellin A_{14} has been available to us for a repetition of this comparison.

Gibberellin A_{14} and gibberellin A_{12} serve as precursors of gibberellic acid in *G. fujikuroi* cultures as shown by Cross and Norton.[17,18] Thus, a role was suggested for those C_{20}

[13] M. KATSUMI, B. O. PHINNEY, P. R. JEFFERIES and C. A. HENRICK, *Science* **144**, 849 (1964).
[14] M. RUDDAT, A. LANG and E. MOSETTIG, *Naturwissenschaften* **50**, 23 (1963).
[15] B. D. CAVELL, J. MACMILLAN, R. J. PRYCE and A. C. SHEPPARD, *Phytochemistry* **6**, 867 (1967).
[16] J. MACMILLAN, R. J. PRYCE, G. EGLINTON and A. MCCORMICK, *Tetrahedron Letters* 2241 (1967).
[17] B. E. CROSS and K. NORTON, *Tetrahedron Letters* 6003 (1966).
[18] B. E. CROSS and K. NORTON, *Chem. Commun.* 535 (1965).

gibberellins with a methyl group at position 4a as intermediates in the biosynthesis of gibberellic acid, and presumably other C_{19} gibberellins, by the fungus. The growth promoting activity of gibberellin A_{14} in the *dwarf*-1, *dwarf*-2, and *dwarf*-5 maize mutant assays and the cucumber seedling assay supports, but does not prove, the hypothesis that the C_{20} gibberellins have a similar intermediary role in the biosynthesis of the C_{19} gibberellins in flowering plants. In line with this reasoning, the sequence of conversions of carbon skeletons in gibberellin biosynthesis, without reference to the stage of introduction of hydroxyl groups and double bonds in the ring system would be formulated as:



(−)-kaurene C_{20} gibberellins C_{19} gibberellins

This hypothesis predicts that the contraction of the B ring occurs prior to the elimination of the C-20 methyl.

EXPERIMENTAL

Isolation of Gibberellin A_{14}

Fusarium Moniliforme. (Strain Lilly M119) was grown for two weeks in 250 ml potato-dextrose liquid medium while maintained on a rotary shaker. This culture was used to inoculate 35 l. of the same medium in a 40-l. carboy. The large culture was grown for 2 weeks under aseptic, aerobic conditions at laboratory temperature. At the completion of the growth period, the cells were separated from the medium in a Sharples Continuous Flow Centrifuge and discarded. The ethyl acetate-soluble organic acids were extracted from the medium according to the scheme outlined in Fig. 3.

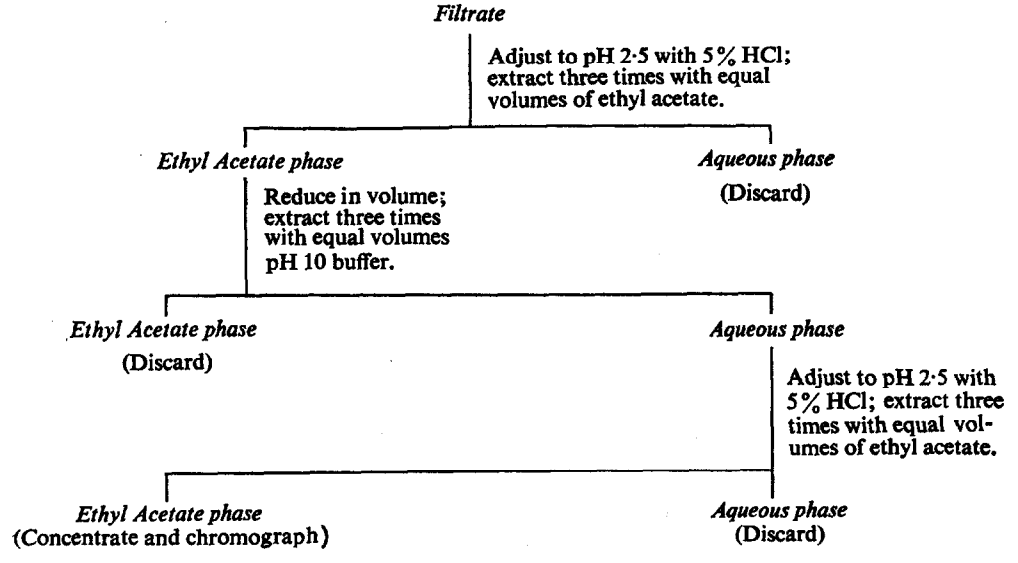


FIG. 3. SCHEME FOR THE ACID-BASE EXTRACTION OF ETHYL ACETATE-SOLUBLE ORGANIC ACIDS.

A buffered Celite column was prepared by mixing 65 ml 1 M phosphate buffer, pH 6·2, and 175 g Celite, and packing the mixture in a 3 cm × 100 cm chromatography column filled with Skelly Solve B (light petroleum, b.p. 60–80°). The mixture of extracted organic acids was added to the column which was then developed by gradient elution according to the method of Hirsch and Ahrens.[19] The lower flask of the gradient elution apparatus initially contained 1 l. Skelly Solve B and the upper flask contained a mixture of 500 ml Skelly Solve B and 500 ml ethyl ether. During column development, the upper flask was refilled twice with 1 l. portions of ether. The effluent was collected in 20 ml fractions; portions (0·02 ml) of every third fraction were chromatographed on a silica gel G chromatoplate with isopropyl ether:acetic acid (95:5 v/v). The developed plates were sprayed with an ethanolic solution of H_2SO_4 (95:5 v/v), heated at 110° for 10 min, and viewed under u.v. light.[8] The fractions of effluent ranging from 1600 to 2000 ml were observed to contain a strongly fluorescing component which migrated to R_f 0·37 (R_{GA_3} 1·43) on the TLC plate. These fractions were combined and the solvent evaporated to leave a gummy residue.

A 3 cm × 20 cm chromatographic column was prepared by packing 30 g of dry silicic acid which was then washed with about 3 l. of anhydrous $CHCl_3$ until it appeared uniformly translucent. The residue obtained from the buffered Celite column was adsorbed onto about 1 g silicic acid which was then added to the top of the column. The lower flask of the gradient elution apparatus initially contained 1 l. $CHCl_3$ while the upper flask contained 1 l. ethyl acetate. During development of the column, two additional 1-l. portions of ethyl acetate were added to the upper flask. The effluent was collected in 20-ml fractions and portions of every third fraction were chromatographed in the thin layer system as described above. The fractions containing the effluent ranging from 2200 to 3400 ml were observed to contain the fluorescing component which migrated to R_f 0·37 on TLC. These fractions were combined and the solvent evaporated to leave a powdery residue (121 mg).

A third chromatographic column was prepared by pouring a suspension of 4 g Celite and 4 g charcoal (Takeda Chemical Industries, Osaka, Japan) in 50 ml methanol into a glass column and allowing the adsorbent to settle. The solvent was drained to the surface of the charcoal and the residue from the silicic acid column, adsorbed onto 1 g charcoal, was added. The column was then developed with anhydrous methanol, 5 ml fractions being collected and analyzed as above. The fractions ranging from 35 to 105 ml containing the component of R_f 0·37 yielded 80 mg of a powdery residue which was crystallized twice from an ethyl acetate-light petroleum mixture (final yield of crystals, 68 mg).

Preparation of the Dimethyl Ester

14·5 mg (0·041 mmole) of the crystalline material were dissolved in 4 ml of ethyl ether containing approximately 0·70 mmoles of diazomethane generated from N-methyl-N-nitroso-*p*-toluenesulfonamide.[20] The solvent was allowed to evaporate and the residue was extracted into ethyl acetate. Evaporation of the ethyl acetate yielded about 14·5 mg of a colourless oil. TLC of a portion (5 μg) on silica gel developed with isopropyl ether-acetic acid (95:5 v/v) followed by a H_2SO_4 spray-heat treatment revealed a major fluorescent component at R_f 0·62 (R_{GA_4} 1·48) along with minor fluorescent zones at R_f 0·56 and 0·30. A sample of the methyl ester free of these impurities was obtained by chromatographing the product on a small column of anhydrous silicic acid developed with benzene–ethyl acetate (8:2 v/v). All attempts to crystallize the methyl ester were unsuccessful.

Chemical Assays

The quantitative microdetermination of olefinic bonds was based on a method described by West.[21] Solutions of gibberellic acid, Δ^4-tetrahydrophthallic anahydride and the unknown were prepared in ethyl acetate in accurately determined concentrations. Measured volumes (containing 40 to 50 μg of compound) were transferred to cuvettes and the solvent was evaporated. An accurately measured volume (3·00 ml) of 4·0 mM $KMnO_4$ in water was added to each cuvette and the solutions were allowed to stand for exactly 15 min at which time the absorbancy at 415 nm was measured using 4·0 mM $KMnO_4$ as a reference solution. Gibberellic acid and Δ^4-tetrahydrophthallic anhydride showed an average change in absorbance of 1·93 units per microequivalent of olefinic bond. The unknown showed an average change of $5{\cdot}50 \times 10^{-3}$ absorbance units per μg which corresponds to 0·99 olefinic bonds per micromole, assuming a molecular weight of 348.

A qualitative test to determine the presence of hydroxyl groups was based on a procedure described by Jones.[22] The methyl ester was treated with acetic anhydride and the reaction mixture examined for acetylated derivatives by thin layer chromatography.

Microanalyses were performed in the Microanalytical Laboratory of the Department of Chemistry, University of California, Los Angeles.

[19] J. HIRSCH and E. H. AHRENS, Jr., *J. Biol. Chem.* 233, 311 (1958).
[20] H. J. DE BOER and H. J. BACKER, *Rec. Trav. Chim.* 73, 229 (1954).
[21] C. A. WEST, p. 473 in Plant Growth Regulation, Fourth International Congress on Plant Growth Regulation, R. M. Klein (editor), The Iowa State University Press, Ames, Iowa (1961).
[22] D. F. JONES, *Nature* 202, 1309 (1964).

Physical Measurements

The molecular weight was determined on a Mecrolab Vapor Pressure Osmometer, Model 301A. The machine was standardized against ethanolic solutions of naphthalene.

I.r. absorption spectra were determined with a Perkin Elmer Model 421 Grating Spectrophotometer in either 0·002 ml of 10 per cent solution in tetrahydrofuran or a micropellet composed of a mixture of 0·3 mg of sample in 3 mg KBr. Tetrahydrofuran was purified shortly before use by passing it through an alumina column (Woelm basic, activity grade I) to remove peroxides, followed by distillation over KOH pellets to remove preservative.

U.v. absorption spectra were measured in a Cary Recording Spectrophotometer, Model 14, using a concentration of 1×10^{-4} M sample in 95 per cent ethanol in 1 cm cells.

NMR spectra were determined with a Varian A-60 Analytical NMR Spectrometer. The K salt of the acid was measured at a concentration of 50 mg per 0·5 ml D_2O with tetramethyl silane as an external reference standard. The methyl ester was run in a capillary at a concentration of approximately 4 mg per 50 μg of $CDCl_3$ with tetramethylsilane as an internal reference standard set to 10·00τ.

Mass spectral measurements were made with an Associated Electrical Industry MS-9 Mass Spectrometer by direct insertion of the sample in the ion source. The reference sample for high resolution measurements was tri-(*n*-perfluorobutyl) amine.

Bioassays

Biological activity was measured in dwarf maize assays,[10] the cucumber seedling assay,[11] and the barley endosperm assay.[12] Dwarf segregants of maize (*Zea mays*, mutants *dwarf*-1, *dwarf*-2, and *dwarf*-5) and cucumber seedlings (*Cucumis sativus* L. var. National Pickling, Burpee Seed Co.) in groups of ten, were each treated with 0·01, 0·1, 1·0, 10·0, or 100·0 μg quantities of the newly isolated compound or with gibberellic acid; these compounds were applied in solution in 50 per cent aqueous acetone in the maize assays, and in 95 per cent ethanol in the cucumber assay. Control groups of ten seedlings were treated with solvent alone in each assay. Relative biological activity was determined in each assay from the ratio of the weight of gibberellic acid required for a given response to the weight of the new gibberellin required for an equal response.

For the barley endosperm assay, three embryo-less barley seed halves (var. White Naked Atlas) were incubated in vials containing 1 ml distilled water, 200 μg streptomycin, and 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} or 10^{-10} g gibberellic acid or new gibberellin. The seed halves were incubated for 48 hr at 27°. The incubation mixture was diluted to 11 ml with distilled water and shaken for 5 min with about 1 g Amberlite 120 (acid form). Three ml of each solution was then mixed with 3·0 ml color reagent, the solution was heated at 100° for 5 min and the absorbance at 540 nm was read in a Bausch and Lomb Spectronic 20 Colorimeter, using diluted color reagent as a standard. The color reagent was prepared by adding 1 g 3, 5-dinitrosalicylic acid in 20 ml 2 N NaOH and 50 ml water, followed by the addition of 30 g potassium sodium tartrate and dilution of the solution to 300 ml with distilled water.

Acknowledgements—We are indebted to Mr. Mike Haugh for his assistance in determining spectra; Miss Heather King, for her assistance in performing microanalyses; and to Mrs. Josephine Liotta, for her assistance in performing bioassays. We are also indebted to Dr. B. E. Cross, University of Leeds, for supplying us with a sample of GA_{14} and for comparing the i.r. spectra of the two compounds, to Dr. J. MacMillan, University of Bristol, for comparing gas chromatographic properties of the two compounds and to Dr. C. W. Schaller, University of California, Davis, for supplying us with barley seed.