lactoside have very similar R_f values, and only separate distinctly when the paper is developed for at least 24 hours with a solvent mixture based on *n*-butanol.

A pelargonidin-3-rhamnoglucoside, probably the 3-rutinoside, previously identified in pink forms of *Antirrhinum majus*[4], has also been found in *Solanum phureja*. The 3-bioside occurring in petals of *Papaver spp.*[8] has been identified as a 3-diglucoside. It is probably the 3-gentiobioside, since it occurs with mecocyanin (cyanidin-3-gentiobioside[9]) in *Papaver rhoeas*. Two further pelargonidin derivatives, one found in *Tritonia*, variety 'Prince of Orange', and the other in *Primula sinensis*[10], differ chromatographically from the above pigments, but must also be considered to be a 3-rhamnoglucoside and a 3-diglucoside respectively. These two cases of isomeric forms of the same glycoside are presumably due to difference in the linkage between the sugar residues of the disaccharides concerned. Indeed, assuming that the combined glucose has the β-D-pyranoside configuration, four isomeric 3-diglucosides of pelargonidin may theoretically occur in nature.

The structure of the pelargonidin-3-rhamnoglucosido-5-monoglucoside of *S. phureja* mentioned previously has been confirmed by identifying the 3-monoglucoside, the 3-rhamnoglucoside, the 3:5-diglucoside and the 5-monoglucoside of pelargonidin as products of its partial acid hydrolysis. Another example of this new type of glycoside is the pigment present in the skin of radishes, *Raphanus sativum*. It is an acylated derivative of pelargonidin 3-diglucosido-5-monoglucoside. On partial hydrolysis, it gives the 3-gentiobioside, the 3:5-diglucoside and the 3- and 5-monoglucosides. Two other novel types of pelargonidin glycoside must be mentioned. One of these occurs in *Primula sinensis*[10], with the 3-monoglucoside and a 3-diglucoside of pelargonidin and appears to be a 3-triglucoside, since only the first two simpler pigments can be detected during acid hydrolysis. Although flavonols with three sugar residues attached at a single position (i.e. the 3-position) have recently been found in nature[11], no anthocyanins of this type have been recorded before.

The other novel glycoside occurs with pelargonidin-3-gentiobioside in *Papaver orientale*. It is remarkable in being distinctly lighter orange in colour than any of the other naturally occurring glycosides of pelargonidin. Indeed, its maximum in the visible spectrum is 499 mμ when measured in methanol, containing a trace of hydrochloric acid. Other pelargonidin glycosides have λ_{max} at 505 mμ in the same solvent. On acid hydrolysis, it gives only glucose and pelargonidin. Four non-fluorescent glycosides are produced during this hydrolysis. Two were identified, namely the 3-gentiobioside and the 3-monoglucoside. It follows that the original glycoside must have two glucose residues in the 3-position and one in the 7- or 4'-position. Up to now, it has always been assumed that anthocyanins only contained sugars substituted in the 3- and 5-positions.

Other results indicate that a similar range of glycosides of the other five commonly occurring anthocyanidins are present in nature. For example, different forms of the cultivated potato are pigmented with the six common anthocyanidins as the 3-diglycosido-5-monoglycoside acylated with *p*-coumaric acid. Although some details of the structure of these pigments remain to be determined, it is apparent from these preliminary results that anthocyanins occur as a range of glycosidic forms comparable with the variation encountered in the flavonol series[12].

The co-occurrence of pelargonidin mono-, di- and triglycosides in *Primula sinensis*, and of di- and triglycosides in *Papaver orientale* and *Solanum phureja* is significant. It appears from these and other results that a number of biosynthetic steps are involved in the glycosidation of anthocyanidins in nature. Genetical evidence supports this view since single gene differences have been related to changes in the glycosidic pattern of the anthocyanins in some plant species[13]. It seems, then, that single sugar residues are linked one at a time to the pigment molecule or precursor rather than that a preformed di- or trisaccharide is attached directly in one step.

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John Innes Horticultural Institution, Bayfordbury, Hertford (Herts), August, 28, 1957.

Zusammenfassung

Neun Glykoside des Pelargonidins wurden durch papierchromatographische Methoden gekennzeichnet. Es war notwendig, die Methode der Zuckeridentifizierung abzuändern, um die Bildung des Kunstprodukts Arabinose zu vermeiden. Einige der neun Glykoside gehören zu neuen Sorten von Anthocyanidinglykosiden, die bisher noch nicht in der Natur entdeckt waren. Die dadurch erklärte Variation im Glykosidformelbild der Anthocyane wird in bezug auf ihre Biosynthese diskutiert.

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Gibberellenic Acid, a By-product of Gibberellic Acid Fermentation

Recently, CROSS *et al.*[1] have proposed a structure (I) for gibberellic acid and have shown that the products of successive acid degradation, *allo*-gibberic and gibberic acid, possess the structures II and III. In our laboratories gibberellic acid, isolated[2] from cultures of *Fusarium moniliforme* and crystallized from ethyl acetate, was found to contain varying amounts of a by-product readily detectable by means of its strong absorption in the ultraviolet region as well as by its immobility on paperchromatogram. Using a butanol-ammonia system[2], for example, it remains at the point of application while gibberellic acid moves with an R_f value of 0·4. This by-product, for which we propose the name gibberellenic acid, was first obtained as a molecular complex with 2

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moles of gibberellic acid. Digestion of this complex with hot chloroform followed by recrystallization of the insoluble residue from ethyl acetate gave gibberellenic acid (m.p. 185°, gas evolution), a dicarboxylic acid with the same composition as gibberellic acid (Analysis. Calculated for $C_{19}H_{22}O_6$: C, 65·88; H, 6·40; mol. weight, 346. Found: C, 66·16; H, 6·58; mol. weight by electrometric titration, 354). In the pea elongation test[3] gibberellenic acid was found to be devoid of biological activity.

In contrast to gibberellic acid which is transparent between 220 and 400 mμ, gibberellenic acid strongly absorbs at 253 mμ (ε = 19,200). Sodium borohydride in methanolic solution does not affect this absorption maximum, but 2 moles of hydrogen are taken up rapidly when gibberellenic acid is reduced with palladium catalyst in ethanol. The resulting solution has become transparent at 253 mμ. With platinum oxide as the catalyst a third mole of hydrogen is taken up slowly.

Gibberellenic acid appears to be an intermediate in the conversion of gibberellic acid to *allo*-gibberic (and gibberic) acid as evidenced by the following observations. Solutions of I in dilute hydrochloric acid (0·1–1 *N*) or in methanolic sulfuric acid at room temperature develop absorption at 253 mμ. In 1% methanolic sulfuric acid this absorption attains in about 6 h a value corresponding to a 90% conversion to gibberellenic acid

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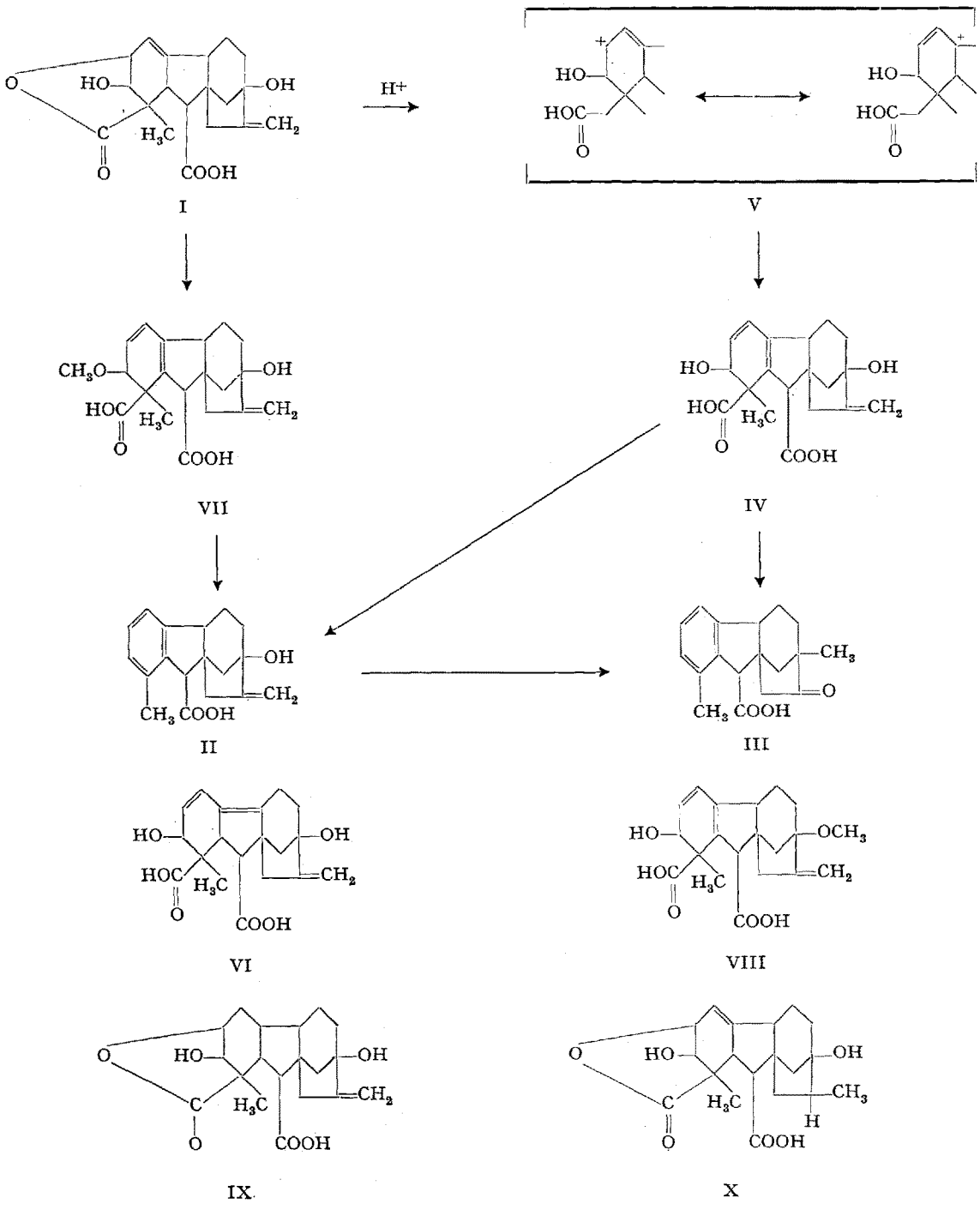

and then remains at this level. In aqueous solution under optimum conditions the maximum value attained corresponds to approximately a 50% conversion and then slowly decreases until the absorption approaches that of *allo*-gibberic acid (max. = 266 mμ, ε = 320)[4]. Furthermore, gibberellenic acid yields *allo*-gibberic and gibberic acid when treated under the acidic conditions described[4] for the preparation of these two acids from I.

The above observations strongly indicate that gibberellenic acid is an artifact produced from I by acid catalysis during the fermentation and/or isolation process. Accepting the proposed structure[1] for gibberellic acid (I), gibberellenic acid may be formulated as IV, its formation from I presumably proceeding by way of the carbonium ion intermediate (V)[5]. No strictly analogous model for comparison of ultraviolet absorption appears to be available but the observed absorption of IV is not incompatible with the values reported for diene systems[6]. The resistance of the tetra-substituted double bond in IV towards hydrogenation (see above) is not surprising and finds its parallel in the observed resistance to catalytic reduction of an analogous double bond in $\Delta^{8,9}$-bicyclo(4·3·0)nonene-5,6-dicarboxylic[7]. Although the evidence presented here does not rule out the alternate structure (VI) for gibberellenic acid, the ready conversion of this acid by decarboxylation and dehydration to the aromatic *allo*-gibberic acid (II) supports structure IV.

Although the data reported here support the contention that gibberellenic acid (IV) is derived from I by acid catalysis, the conversion of I to IV in aqueous acid on a preparative scale was not feasible because of the low solubility of I and the formation of further degradation products under these conditions. On the other hand, acid methanolysis followed by neutralization with methanolic sodium methoxide provided a new crystalline dibasic acid (m.p. 188–190°, gas evolution) which likewise has an absorption maximum at 253 mμ (ε = 19,000). This acid was recovered unchanged after a 12 h treatment with 1 *N* sodium hydroxide. This observation together with the analytical data (Calculated for $C_{20}H_{24}O_6$: C, 66·65; H, 6·71; O–CH_3(1), 8·61; mol. weight, 360. Found: C, 66·11; H, 7·25; O–CH_3, 7·89; mol. weight by electrometric titration, 360) allow the formulation of this acid as the methyl ether of gibberellenic acid (VII). The alternate structure (VIII) can be ruled out since its formation requires an unlikely carbonium ion intermediate. That this methyl ether-acid is correctly represented by VII was demonstrated by its conversion to *allo*-gibberic acid (II), employing the mild acidic conditions[4] which effect the conversion of I to II. Vigorous acid treatment[4] of VII yields gibberic acid (III). The direct conversion of IV to VII using methanolic sulfuric acid could not be realized indicating that the conversion of I to VII does not involve IV as an intermediate.

Gibberellin A_1 has been reported[8] to be a dihydrogibberellic acid having one double bond and should therefore be IX or X. As a sample of gibberellin A_1[9] in acidic solution did not develop the typical absorption at 253 mμ, it must possess structure IX. This same structure has recently been proposed by SUMIKI *et al.*[8] on the basis of ozonolysis experiments.

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The Lilly Research Laboratories, Indianapolis, Indiana, July 30, 1957.

Zusammenfassung

Unter den Fermentationsprodukten von *Fusarium moniliforme* findet sich neben der bekannten Gibberellinsäure als Nebenprodukt die Gibberellensäure. Identifizierung und Charakteristika dieser Säure (IV) werden beschrieben. Für Gibberellin A_1 wird die Struktur (IX) angegeben.

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Release of Bradykinin as Related to the Esterase Activity of Trypsin and of the Venom of *Bothrops jararaca*

In a previous paper[1] we have shown that the bradykinin releasing activity of the venom of *Bothrops jararaca* is not related to its proteolytic activity on casein. Two apparently distinct proteolytic activities were demonstrated in the venom depending upon their different heat tolerance. While the activity on casein was found to be easily destroyed on a few minutes heating in boiling water, the remaining esterase activity on benzoyl-L-arginine methyl ester (BAME) proved to be more thermostable and only partially destroyed[2]. As shown by SCHWERT *et al.*[3] trypsin besides its proteolytic activity on casein also displays esterase activity on BAME, the latter being correlated to its amidase activity on benzoyl-L-arginine amide[4]. The venom of *Bothrops jararaca* has also been shown to act on casein and the benzoyl-L-arginine ethyl ester[5]. It therefore seems possible that the common mediator for bradykinin release of both trypsin and the snake venom may be their respective esterase activities. In the present work we have shown that such a correlation can be demonstrated. If trypsin inhibitor is removed by applying the denatured plasma as a substrate a direct relationship is found between the bradykinin releasing effect of trypsin and the heated venom of *Bothrops jararaca* when compared with their respective esterase activities on BAME.

Heating a solution of the venom in a boiling water bath for 1–3 min[1] was enough to destroy completely its

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