Acknowledgment.—The authors wish to thank Mr. Louis Brancone and staff who performed the microanalyses and Anna de Grunigen who carried out the spectra determinations.

Summary

Descriptions are given for the synthesis of 2-amino-4-hydroxy-7-methylpteridine (III) from 2,4,5-triamino-6-hydroxypyrimidine (II) and each of the following: 1,3-dichloroacetone, 2,3-dichloropropional, α-bromotetronic acid and *d,l*-glyceraldehyde.

The synthesis of 2-amino-4-hydroxypteridine-6-acetic acid from 2,4,5-triamino-6-hydroxypyrimidine and ethyl 2,4-dibromo-3-ketobutanoate is described and a mechanism for these reactions is suggested.

PEARL RIVER, NEW YORK RECEIVED APRIL 1, 1948

[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

A Flavonone from Douglas-Fir Heartwood[2]

BY JOHN C. PEW

A new compound has been found at the U. S. Forest Products Laboratory in Douglas-fir heartwood. This compound may be responsible for the resistance to sulfite pulping shown by the heartwood of this species. Erdtman[3] found a 3,5-dihydroxystilbene and its monomethyl ether in pine heartwood and showed these substances caused sulfite-pulping retardation. Douglas-fir heartwood, when subjected to Erdtman's method of separation, did not yield stilbene derivatives. Staining reactions, however, showed the presence of phenolic substances, and the crystalline compound isolated gave similar reactions.

The compound, accompanied by considerable extraneous matter, is extracted from the wood by methanol, ethanol, acetone or ethanol–benzene mixtures. Ether does not extract it, but dissolves it after extraction. Separation of the pure substance from the other extractives is somewhat difficult. The compound crystallizes as a hydrate from water in long, fine, colorless needles that melt with decomposition at 240–242° (dec.).[4] It is optically active, giving a value of $[\alpha]^{20}D + 46°$ (*c*, 4 in equal volumes of acetone and water) and $[\alpha]^{20}D + 13°$ (*c*, 4 in absolute alcohol). With ferric chloride it gave emerald-green to black colorations in aqueous solution, and with magnesium and hydrochloric acid it gave an intense purple-red color in alcoholic solution. This latter reaction suggested the structure of a flavanone. Atmospheric oxidation on a steam-bath of a 2 *N* sulfuric acid solution of the compound gave good yields of quercetin. Reduction of an alcoholic solution with zinc dust and hydrochloric acid gave the flavanone eriodictyol. Heating the compound with strong hydrochloric acid produced a compound with no optical activity. From these properties and the analysis the compound was formulated as 3,3′,4′,5,7 pentahydroxyflavanone.[5]

(1) Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

(2) Presented before the American Chemical Society, New York, N. Y., Sept. 15–19, 1947.

(3) H. Erdtman, *Ann.*, **539**, 116 (1939).

(4) All melting points were corrected.

(5) Erdtman (private communication) suggests the compound be called "taxifolin."

The 3-hydroxyflavanones (or flavanolones)[6] belong to a class found in recent years in plants. The first example, designated alpinone,[7] and shown to be the 3,5-dihydroxy-7-methoxy-2-methylflavanone, was discovered in 1936 in a Japanese drug prepared from *Alpinia japonica*. Subsequently, three other 3-hydroxyflavanones were reported to occur naturally: fustin[8] (3,3′,4′,7-tetrahydroxyflavanone) from the heartwood of the *Rhus* sp.; ampeloptin[9] (3,3′,4′,5,5′,7-hexahydroxyflavanone) from *Ampelopsis meliaefolia* Kudo, a plant used as a drug and condiment in China; and pinobanksin[10] (3,5,7-trihydroxyflavanone) from the heartwood of *Pinus banksiana* and other *Pinus* sp. The presence of the 3-hydroxyflavanones in the heartwood of two species suggested the possibility of their occurrence in other woods. A South American wood, coigue (*Nothofagus dombeyi* Blume), the heartwood of which showed resistance to sulfite pulping, yielded naringenin and the corresponding 3-hydroxy compound, both of which gave cherry-red reduction colors with magnesium and hydrochloric acid. These two flavanones were also isolated from the heartwood of black cherry (*Prunus serotina* Ehrh). The 3-hydroxyflavanones were found to have a color reaction that appears to be characteristic. Their alcoholic solutions give deep anthocyanidin-like colors when treated with granulated zinc and hydrochloric acid, while the corresponding flavanones without the 3-hydroxy group and the flavanols, under similar treatment, remain colorless or give only weak pink to lavender tints. Both classes of flavanones readily give deep colors with magnesium and hydrochloric acid. If the filtrate from suspensions of ground wood (or other plant material) in methyl alcohol, after standing in contact a day or two, are not colored when acidified with hydrochloric acid but develop distinct colors on the subsequent addition of granulated zinc, the

(6) H. Erdtman, *Svensk Kem. Tid.*, **56**, 8 (1944).

(7) Y. Kimura and M. Hoshi, *Proc. Imp. Acad., Tokyo*, **12**, 285 (1936).

(8) T. Oyamada, *Ann.*, **538**, 44 (1939).

(9) M. Kotake and T. Kubota, *Ann.*, **544**, 253 (1940).

(10) H. Erdtman, *Svensk. Kem. Tidskr.*, **56**, 8 (1944).

presence of 3-hydroxyflavanones is to be suspected. Thus American beech heartwood (*Fagus grandiflora* Ehrh.) gives a cherry-red color when treated in this manner, due probably to the same 3-hydroxyflavanone that occurs in coigue, although the concentration appears to be much lower in the beech.

In addition to its close relationship to quercetin and eriodictyol, the Douglas-fir flavanone bears a resemblance to catechin. Like catechin, it contains two dissimilar asymmetric carbon atoms and probably can exist in two *d*, *l*, and *dl* forms. Only a *d* form was actually isolated. Attempts were made to convert the flavanone to catechin by Clemmensen reduction. Crystalline catechin was not isolated, but amorphous substances were produced that gave phlobaphene-like precipitates with boiling mineral acids and also strong wood-splint reactions for the phloroglucinol nucleus (as does catechin and phloroglucinol tannins, but not the original flavanone).

A number of attempts were made to prepare the flavanone before a successful method was developed. The literature[11] recorded the synthesis of the 3-hydroxy-3′,4′,5,7-tetramethoxyflavanone, but the myricetin used to prepare the phenone required for this synthesis was unavailable and, from the observed action of hydriodic acid on the Douglas-fir flavanone, successful demethylation of the compound appeared dubious. Various modifications of the direct condensation of ω-hydroxyphloracetophenone and protocatechualdehyde in the cold[12] and hot[13] failed to produce the flavanone or corresponding chalcone. Condensation of the benzoylated phenone and aldehyde with dry hydrogen chloride[14] also was unsuccessful. The literature[15] recorded the preparation of the 4′-methoxy derivative of the desired compound by bromination of tetraacetylhesperitin and subsequent removal of the bromine with silver acetate followed by hydrolysis of the pentaacetate produced. One attempt was made to employ this procedure, using eriodictyol as the starting material. The desired 3-hydroxyeriodictyol was not obtained, but the method appeared promising. Confirming a previous report in the literature,[16] hydrogenation of quercetin with platinum catalyst was unsuccessful, as the hydrogen remained unadsorbed. Hydrogenation with palladium-on-charcoal gave the same result. Hydrogenation of quercetin in methanol solution over Raney nickel at 100° for fifteen minutes produced traces of the compound, but the yield could not be built up by lengthening the time or altering other conditions. It was found, however, that quercetin in methanol solution could be reduced to the flavanone by long treatment (several days) with tin and hydrochloric acid. Yields at best were only about 7%. Finally, it was discovered that quercetin could be reduced in an aqueous sodium carbonate solution with sodium hydrosulfite at 100°.

The amount of flavanone present in Douglas-fir heartwood is variable and difficult to determine accurately because of the considerable losses in purification. In one sample 2.2% of crude flavanone was obtained. The usual amounts are generally smaller, probably in the neighborhood of 1% of the pure anhydrous compound.

Because of the presence of the 3-hydroxyflavanones in the Asiatic drugs mentioned above and from their relationship with structures recently discussed in the literature in connection with "Vitamin P" and antihemorrhagic substances, it is suggested that the Douglas-fir or cherry compounds might have pharmaceutical value.

Experimental

Extraction and Separation of the Douglas-fir Flavanone.—Air-dry Douglas-fir heartwood (2.38 kg., equivalent to 2.0 kg. moisture free), ground to pass the 1-mm. perforations in a Wiley mill, was added to 20 liters of a mixture of equal volumes of methyl alcohol and water. After the suspension was stirred for seventy-two hours, the solution was filtered off and the filter cake pressed out. The extract was concentrated under reduced pressure to a volume of about 1 liter, the amorphous precipitate filtered off, and the filtrate extracted with four 50-cc. portions of chloroform; the extract was discarded and the filtrate then extracted with one 300-cc. and six 100-cc. portions of ether. The ether extracts were combined and dried over anhydrous sodium sulfate, and the ether was distilled to a volume of about 100 cc. Petroleum ether (50 cc.) was added and the mixture set aside to crystallize. When the precipitated material had solidified, 450 cc. more of petroleum ether was added and the solid broken up with a stirring rod. After standing for twenty-four hours, the residue was filtered off, washed with petroleum ether, and dried for four hours in a vacuum oven at 105°.

The yield was 12.4 g. of flesh-colored crystalline material, m. p. 228–231° (dec.), yield, corrected for loss of solution in press cake, 14.3 g. Recrystallization from 50% aqueous ethyl alcohol gave light cream-colored needles of nearly pure material, m. p. 236–238° (dec.). Repeated crystallization gave long, thin colorless needles, m. p. 240–242° (dec.) in Pyrex capillaries.[17] Other flavanones, apparently naringenin and 3-hydroxynaringenin, were indicated in some samples of Douglas-fir, but only in relatively small amounts.

Anal. Calculated for $C_{15}H_{12}O_7$: C, 59.21; H, 3.95. Found: C, 59.08; H, 4.00.

Properties.—The flavanone was found to be readily soluble in alcohols, acetone, acetic acid and boiling water, somewhat soluble in ether and in cold water, and practically insoluble in benzene. Its aqueous solution gave an emerald green to black coloration with ferric chloride, depending on concentration. In alcoholic solution, ferric chloride gave a blackish orange and uranium acetate a clear orange color. A solution in dilute sodium hydroxide was nearly colorless at first, but quickly became brown on exposure to air. The Wilson boric acid test for flavanone derivatives[18] was negative in the pure material. An alcoholic solution treated with hydrochloric acid and zinc, magnesium or sodium amalgam gave a deep purple-red color, similar to that obtained with eriodictyol solution, magne-

(11) Y. Kimura, *J. Pharm. Soc. Japan*, **58**, 415 (1938).
(12) E. F. Kurth, THIS JOURNAL, **61**, 861 (1939).
(13) L. Reichel, W. Burkart and K. Müller, *Ann.*, **550**, 146 (1942).
(14) A. J. Russell, *J. Chem. Soc.*, 421 (1937).
(15) G. Zemplén and R. Bognar, *Ber.*, **76B**, 452 (1943).
(16) R. Mozingo and H. Adkins, THIS JOURNAL, **60**, 669 (1938).

(17) Soft glass capillaries were unsuitable even when washed with acid after drawing. Premature browning occurred at the glass surface, and a definite depression in melting point was observed. Several other flavanones behaved similarly.
(18) C. W. Wilson, THIS JOURNAL, **61**, 2303 (1939).

sium and acid. The solution reduced with zinc became orange when it was made alkaline with sodium hydroxide, while the solution reduced with sodium amalgam became bluish purple to violet depending on concentration. On fusion, the flavanone decomposed to a reddish-brown mass. When the material was heated in air until just liquified, then quickly cooled, quercetin, identified by its conversion to bromoquercetin, m. p. 235–237° (literature[19] 236–237°) was precipitated out by the addition of water.

Lead Salt of the Flavanone.—The flavanone (2 g.) was dissolved in 400 cc. of boiling water, 1 cc. of acetic acid was added, the heating was discontinued, and 45 cc. of a 10% solution of lead acetate was slowly added with constant stirring. After digestion for an hour the crystalline precipitate was filtered off, thoroughly washed with hot water, and dried at 105° for two hours.

Anal. Calculated for $C_{15}H_{10}O_7Pb$: Pb, 40.7; for $C_{15}H_{11}O_7 \cdot PbOH$: Pb, 39.3. Found: Pb, 39.3.

Water of Hydration.—The flavanone was crystallized from 20 and also from 50 parts of hot water and the crystals dried several days at 27° and 65% relative humidity. Weighed portions were then oven-dried at 120° to constant weight.

Anal. Calculated for $C_{15}H_{12}O_7 \cdot H_2O$: H_2O, 5.59. Found (crystals from 20 parts water) H_2O, 5.67. Calculated for $C_{15}H_{12}O_7 \cdot 2.5H_2O$, 12.89. Found (crystals from 50 parts water): H_2O, 12.93.

Oxidation to Quercetin.—The Douglas-fir flavanone (1.00 g.) was added to 100 cc. of 2 *N* sulfuric acid and heated on the steam-bath under a reflux condenser while a gentle stream of air was passed into the flask over the liquid. (Passing air into the liquid through a gas diffusion tube gave poor yield due to extensive decomposition into amorphous products.) In a short time the solution became yellow, and after several hours yellow needles began to precipitate. After twenty-seven hours the solution was filtered, the filtrate returned to the apparatus, and the precipitate thoroughly washed with cold water, then dried at 120°, in a vacuum oven. The yield was 0.374 g. of bright yellow needles, m. p. 316–318° (dec.) (literature[20] 316–317°) unchanged on admixture with an authentic specimen of quercetin. The filtrate, light amber in color, was subjected to a second twenty-seven-hour treatment and yielded 0.248 g. of slightly discolored yellow needles, m. p. 314–316° (dec.). A third treatment yielded 0.147 g. of brown crystalline material, which, on recrystallization from 50% ethanol, gave 0.135 g. of a yellow crystal powder, m. p. 314–316. Further treatment of the acid filtrate yielded only traces of quercetin. The total yield of nearly pure quercetin was 0.757 g., 76% of theoretical. The quercetin was further characterized by preparation of bromoquercetin melting at 235–237° (literature[19] 236–237°).

Attempts to bring about dehydrogenation of the flavanone with other oxidizing agents or by catalytic means were unsuccessful.

Anal. Calculated for $C_{15}H_{10}O_7$: C, 59.60; H, 3.31. Found: C, 59.72; H, 3.47.

Racemization of the Douglas-fir Flavanone.—The naturally occurring compound gave an optical rotation of $[\alpha]^{20}D$ +46° (*c*, 4 in equal volumes acetone and water) and $[\alpha]^{20}D$ +13° (*c*, 4 in absolute alcohol). The flavanone (1 g.) was added to 100 cc. of a mixture of equal volumes of concd. hydrochloric acid and water, refluxed fifteen minutes, diluted to 500 cc. with water, and extracted with one 200-cc., then three 100-cc. portions of ether. The ether extract was washed with two 10-cc. portions of water and the ether distilled off. The residue was dissolved in 65 cc. of water and allowed to remain overnight. There was obtained 0.72 g. of pale, tan-colored, very thin, glistening lens-shaped plates, m. p. 236–238° (dec.). The material was less soluble in water than the original flavanone, but gave the characteristic zinc and hydrochloric acid reduction color. On repeated recrystallization the plates became elongated hexagons, showed no optical activity, and had a m. p. of 240–242° (dec.). The mixed m. p. with the original flavanone was also 240–242° (dec.).[21]

Anal. Calculated for $C_{15}H_{12}O_7$: C, 59.21; H, 3.95. Found: C, 59.43; H, 4.09.

Reduction of Douglas-fir Flavanone to Eriodictyol.—The flavanone (2.00 g.) was dissolved in 20 cc. of methanol, and 4 g. of zinc dust was added. While vigorous agitation of the suspension was maintained, 10 cc. of concd. hydrochloric acid was added in 1-cc. portions over a period of thirty minutes. An additional 2 g. of zinc dust was then added, followed by 5 cc. more of hydrochloric acid over a second thirty-minute interval. Stirring was continued for a third thirty-minute period, the excess zinc filtered off, and 400 cc. of cold water added to the filtrate. Crystals appeared in several minutes. The mixture was allowed to stand overnight at 5°. After filtration, the crystals were washed with water and dried at 120° in a vacuum oven. The yield was 0.91 g. of buff-colored material, m. p. 260–266° (dec.). The filtrate was extracted with one 200-cc. and then two 100-cc. portions of ether. The ether was evaporated, and the residue crystallized from water, yielding 0.13 g. of pink crystals, m. p. 258–266° (dec.). The total yield was 1.04 g., or 55% of theoretical. On recrystallization from 50% aqueous ethyl alcohol, nearly colorless needles, m. p. 270–272° (dec.) were obtained (literature 267°[22]). The material gave a purple-red color when treated in alcoholic solution with hydrochloric acid and magnesium, but not when treated with zinc.

Anal. Calculated for $C_{15}H_{12}O_6$: C, 62.50; H, 4.17. Found: C, 62.52; H, 4.27.

When acetylated by refluxing for six hours with acetic anhydride and sodium acetate,[23] eriodictyol tetraacetate (colorless prisms, m. p. 137–141°, literature[24] 137° sinters, 141° melts)[25] was produced.

Anal. Calculated for $C_{15}H_8O_2(OCOCH_3)_4$: CH_3CO, 37.7. Found: CH_3CO, 38.5.

In addition a white powder m. p. 201–202°[26] was formed.

Conversion of Quercetin to the Racemic Form of Douglas-fir Flavanone.—Quercetin (2.0 g.) was ground with 17 g. of sodium carbonate and the mixture dissolved, with heating, in 200 cc. of water. Sodium hydrosulfite (40 g., 90% pure) was added and the solution immersed in a boiling water-bath for fifteen minutes. Hydrogen sulfide was evolved as the solution lightened in color, and a yellow precipitate formed. The mixture was diluted with 250 cc. of water and cooled, and hydrochloric acid (1 part concentrated acid in 4 parts water) was added until the fugitive orange color of the free hydrosulfurous acid remained for a short time. The mixture was set aside for four hours to precipitate unchanged quercetin, the quercetin filtered

(19) C. Liebermann, *Ber.*, **17**, 1683 (1884).

(20) F. Mayer, "The Chemistry of the Natural Coloring Matters," A. C. S. Monograph No. 89, 1943, p. 188.

(21) The active compound apparently racemizes at the melting point. A sample heated to the sintering point, quickly cooled, then crystallized from water gave, in part, the typical lens-shaped plates of the racemic material.

(22) F. Mayer, "The Chemistry of the Natural Coloring Matters," A. C. S. Monograph No. 89, 1943, p. 184.

(23) L. Reichel, W. Burkart and K. Müller, *Ann.*, **550**, 150 (1942).

(24) G. Zemplén, R. Bognar and L. Szego, *Ber.*, **76B**, 1112 (1943).

(25) The eriodictyol is evidently an optically active form, though only very slight rotation (*c*, 1 in parts acetone and water) was noted. The m. p. of the compound is somewhat high, and on mild acetylation (pyridine and acetic anhydride at room temperature) the acetyl compound melted at 118–120°. Refluxing three hours with sodium acetate and acetic anhydride produced both the 137–141° and 118–120° m. p. products. When the Douglas-fir flavanone was racemized as previously described, the m. p. of the eriodictyol produced by reduction was 267° (dec.), and mild acetylation then gave a product melting at 137–141°.

(26) This compound is not due to ring opening since the acetyl content (34.7%) is less than that of the tetraacetate. It is probably the result of a Perkin-type condensation of an additional acetyl group with the carbonyl of the flavanone similar to that indicated by T. A. Geissman and R. O. Clinton, THIS JOURNAL, **68**, 697 (1946).

off, and the moist precipitate reduced as before by using one-fifth the quantity of reagents. Filtrates from the two reductions were combined, extracted with one 300-cc. and three 200-cc. portions of ether, and the ether extract washed with two 25-cc. portions of water. The ether was distilled off and the residue crystallized from 75 cc. of boiling water. The yield was 0.90 g. of pale-yellow, lens-shaped, thin plates, m. p. 228–232° (dec.), which gave a deep purple-red color on addition of zinc and hydrochloric acid to their alcoholic solution. The yellow substance (possibly the corresponding chalcone) was difficult to remove, and several recrystallizations from water and aqueous alcohol were necessary. The purified material crystallized in elongated hexagonal plates or in needles (the two crystalline forms were interconvertible and probably were two different hydrates), m. p. 238–241° and mixed m. p. with the racemized natural flavanone 238–241° (dec.).

Anal. Calculated for $C_{15}H_{12}O_7$: C, 59.21; H, 3.95. Found: C, 58.85; H, 4.04.

Coigue Flavanones.—Coigue heartwood (*Nothofagus dombeyi*) when processed by the same general methods as described for Douglas-fir, gave a mixture of two flavanones. One, separated by its very low solubility in water, crystallized from aqueous alcohol in colorless needles, m. p. 252–253° (dec.) (literature[27] for naringenin 251°). Mixed m. p. with naringenin 252–253°.

An alcoholic solution gave a cherry-red color with magnesium and hydrochloric acid, but not with zinc and hydrochloric acid.

Anal. Calculated for naringenin $C_{15}H_{12}O_5$: C, 66.18; H, 4.41. Found: C, 65.46; H, 4.62.

The second more soluble flavanone (the 3-hydroxyflavanones appear to be considerably more soluble in water and alcohol than the corresponding 3-desoxy compounds) crystallized from 300 parts of water as a hydrate in the form of colorless needles, m. p. 237–241° (dec.), $[\alpha]^{20}D$ +45° (*c*, 4 in equal volumes acetone and water).

Anal. Calculated for $C_{15}H_{12}O_8$: C, 62.50; H, 4.17. Found: C, 62.60; H, 4.18. Calculated for the hydrate $C_{15}H_{12}O_8 \cdot 1.5H_2O$: H_2O, 8.57. Found: H_2O, 8.37.

Zinc and hydrochloric acid gave a cherry-red color with an alcoholic solution; and ferric chloride, either in aqueous or alcoholic solution, produced a dull orange color. When reduced with zinc dust and hydrochloric acid, as described with Douglas-fir flavanone, the purified crystals, m. p. 249–251° (dec.), showed no depression in m. p. when mixed with an authentic sample of naringenin. When oxidized with air in sulfuric acid solution, yellow spherocrystals, m. p. 270° softens, 279° melts (literature[28] for kaempferol 276–278°), resulted.

Cherry Flavanones.—With cherry heartwood (*Prunus serotina*) two flavanones were isolated that gave undepressed mixed melting points with the respective compounds described under coigue. The whole extract, however, gave a more purplish tint with zinc and hydrochloric acid than the isolated flavanone, indicating one or more additional 3-hydroxyflavanones to be present.

A crystalline material was recovered along with the flavanones that gave no reduction color, but produced an emerald green with ferric chloride, gave a phlobaphene-like substance with boiling mineral acids, and the wood-splint reaction with hydrochloric acid. The hydrate (needles containing 19.8 % water) had a m. p. of 94–96°; anhydrous material m. p. 173–176°, $[\alpha]^{27}D$ +14 (approximately) (*c*, 3 in equal volumes of acetone and water). These properties correspond to *d*-catechin, with which the compound gave an undepressed mixed melting point.

Pulping Studies

Small-scale sulfite pulping experiments were made, using unextracted and extracted Douglas-fir heartwood, Douglas-fir sapwood, spruce with the Douglas-fir flavanone added to the cooking liquor, and spruce impregnated with the Douglas-fir flavanone. The results indicated that the flavanone in Douglas-fir heartwood is an important factor in the resistance of this species to sulfite pulping.

Summary

1. A new flavanone, the 3,3′,4′,5,7-pentahydroxy derivative, was isolated from Douglas-fir heartwood.

2. The flavanone was oxidized to quercetin by air and reduced to eriodictyol with zinc dust and hydrochloric acid. Preparation of the racemic flavanone was accomplished by reduction of quercetin with sodium hydrosulfite.

3. Naringenin and another new flavanone, 3-hydroxynaringenin, were found in coigue and black cherry heartwoods.

(27) F. Mayer, "The Chemistry of the Natural Organic Coloring Matters," A. C. S. Monograph No. 89, 1943, p. 176.

(28) F. Mayer, *ibid.*, p. 182.

MADISON, WISCONSIN RECEIVED MAY 16, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, MEDICAL RESEARCH DIVISION, SHARP AND DOHME, INC.]

Isolation of a Crystalline Trypsin Inhibitor–Anticoagulant Protein from Pancreas[1a]

BY LOUIS A. KAZAL, DANIEL S. SPICER AND ROSE A. BRAHINSKY

The isolation of a crystalline trypsin inhibitor from pancreas was reported by Kunitz and Northrop[1] in 1936. According to their method minced pancreas was treated with dilute sulfuric acid, crystalline chymotrypsinogen and trypsinogen then were separated by fractional precipitation with ammonium sulfate, and trypsin, by precipitation with trichloroacetic acid. The inhibitor, a polypeptide, was crystallized from approximately 0.7 saturated ammonium sulfate solution at *p*H 5.5 and 20° in the form of long hexagonal prisms.

The crystalline inhibitor from pancreas was shown to be an anticoagulant by Ferguson[2] and by Grob[3] inasmuch as the coagulation of recalcified plasma was inhibited *in vitro*.

In the course of other studies in this Laboratory the observation was made that a substance with anticoagulant properties was obtainable from the 15% sodium chloride filtrate that was discarded in the process for the preparation of insulin from

(1a) Presented at the meeting of the American Chemical Society, Division of Biological Chemistry, in New York City, September 16, 1947.

(1) Kunitz and Northrop, *J. Gen. Physiol.*, **19**, 991 (1936).

(2) Ferguson, *Proc. Soc. Exptl. Biol. Med.*, **51**, 373 (1942).

(3) Grob, *J. Gen. Physiol.*, **26**, 423 (1943).