as the main fraction 11.1 g. (19%) of a liquid of b.p. 86–97°. There was a small forerun, and 8.0 g. of black tar remained behind. On redistillation in a molecular still there was obtained a fraction with $n^{25}D$ 1.4903 which gave a positive halogen test, a positive Baeyer test, and a negative Schiff test.[32]

The 2,4-dinitrophenylhydrazone was prepared in the usual manner. After recrystallization from ethanol it had m.p. 218–220° (dec.).

Anal. Calcd. for $C_{10}H_9N_4O_4Br$: N, 17.0; Br, 24.2. Found: N, 16.8; Br, 24.28.

N,N-Diphenylcrotonamide was prepared by a previously described method[33] involving the interaction of crotonyl chloride and diphenylamine in benzene. Recrystallization from dilute ethanol gave shiny colorless crystals, m.p. 114–115° (reported 113–114°,[33] 115–116°[34]). Bromination of the amide with N-bromosuccinimide in carbon tetrachloride in the presence of benzoyl peroxide gave none of the expected product.

N-Crotonylpiperidine was prepared by the same method to give 72% of an oil, b.p. 142.5° (21 mm.), $n^{26}D$ 1.5059, d^{26}_4 1.0006; *MR* obsd. 41.88, *MR* calcd. 44 92.

Anal. Calcd. for $C_9H_{15}ON$: C, 70.55; H, 9.87; N, 9.14. Found: C, 71.58; H, 9.78; N, 8.70.

The dibromide was prepared in good yield by the addition of bromine to a carbon tetrachloride solution of the compound. Recrystallization from dilute alcohol and then from hexane gave stout needles, melting constantly at 96.5–98.0°. The analysis shows the material still contains some impurity.

(32) W. Flaig, *Ann.*, **568**, 24 (1950).
(33) N. Maxim, *Bull. soc. chim. Romania*, **10**, 97 (1928); *C. A.*, **23**, 2697 (1929).
(34) C. Bischoff, *Ber.*, **34**, 2135 (1901).

Anal. Calcd. for $C_9H_{15}ONBr_2$: C, 34.53; H, 4.83; Br, 51.06. Found: C, 34.90; H, 4.66; Br, 49.7.

Attempts to brominate N-crotonylpiperidine with N-bromosuccinimide in carbon tetrachloride with benzoyl peroxide catalyst led to the isolation of 70% of crude succinimide. Distillation *in vacuo* of the product. however, resulted in extensive decomposition and no identifiable product was isolated.

Ethyl γ-phthalimidocrotonate[35,36] was prepared in 70% yield by refluxing equimolar amounts of ethyl γ-bromocrotonate and potassium phthalimide in absolute ethanol for 24 hours. The product after several crystallizations from dilute ethanol and dilute acetone gave shiny plates, m.p. 96–97°.

Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 65.11; H, 5.07; N, 5.40. Found: C, 65.17; H, 4.93; N, 5.4.

Hydrolysis of ethyl γ-phthalimidocrotonate with 6 *N* HCl for 24 hours gave a clear solution from which no solid could be isolated. Treatment of the crude product with phenylacetyl chloride and alkali led to the formation of a solid that after several crystallizations from ethanol is obtained as fine silky needles, m.p. 194.5–196.5°, identified as phenylacetylimide.[37]

Anal. Calcd. for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.81, 75.93; H, 5.71, 5.72; N, 5.45, 5.45.

(35) The corresponding methyl ester has recently been prepared; W. Langenbeck and H. Boser, *Chem. Ber.*, **84**, 526 (1951).
(36) We are indebted to Mr. Alfred Haven for the preparation of this compound.
(37) O. Diels, *Ber.*, **36**, 747 (1903).

AMHERST, MASSACHUSETTS

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

Anthochlor Pigments of *Cosmos sulphureus*, *Coreopsis lanceolata* and *C. saxicola*

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From the ray flowers of *Cosmos sulphureus*, a new anthochlor glycoside, sulphurein, has been isolated, and its constitution established as being the 6-glucoside of sulphuretin (3′,4′,6-trihydroxybenzalcoumaranone). Also isolated from the same plant was coreopsin, previously reported by Geissman and co-workers to be a glucoside of butein (3,4,2′,4′-tetrahydroxychalcone). The structure of coreopsin has now been determined to be the 4′-glucoside of butein. From the ray flowers of *Coreopsis lanceolata* and *C. saxicola*, a new chalcone glucoside, lanceolin, has been isolated. Lanceolin is considered to be the 4′-glucoside of lanceoletin (2′,4′,3,4-tetrahydroxy-3′-methoxychalcone). Leptosin, previously obtained by Geissman from *C. grandiflora*, has now also been obtained and identified from *C. lanceolata* and *C. saxicola*. Leptosin is the 6-glucoside of leptosidin (3′,4′,6-trihydroxy-7-methoxybenzalcoumaranone).

Introduction

Geissman and co-workers[1] isolated coreopsin, a butein glycoside, from the ray flowers of *Coreopsis gigantea* and *Cosmos sulphureus*, and leptosin, a glycoside of leptosidin, from the ray flowers of *Coreopsis lanceolata*.

We have also been, independently, engaged since 1948 in the study of yellow coloring matters in the flowers of some species of Coreopsis which are cultivated in Japan, such as *C. lanceolata L.*, *C. saxicola* Alexander and *Cosmos sulphureus Cavière*. We had isolated from the former two plants a chalcone glycoside and also an orange-yellow glycoside, and from the latter plant a butein glycoside and also an orange-yellow glycoside. Because of the shortage of foreign literature, which lasted from the outbreak of the War until several years after its end, we were unable to notice the works of Geissman until early in 1950. On reading the articles of Geissman, we became convinced that the butein glycoside from *Cosmos* and the orange-yellow glycoside from *Coreopsis*, isolated by us, are identical with coreopsin and leptosin, respectively.

(1) T. A. Geissman, THIS JOURNAL, **63**, 2689 (1941); **64**, 1704 (1942); T. A. Geissman and C. D. Heaton, *ibid.*, **65**, 677 (1943); **66**, 486 (1944); T. A. Geissman and W. Mojé, *ibid.*, **73**, 5765 (1951).

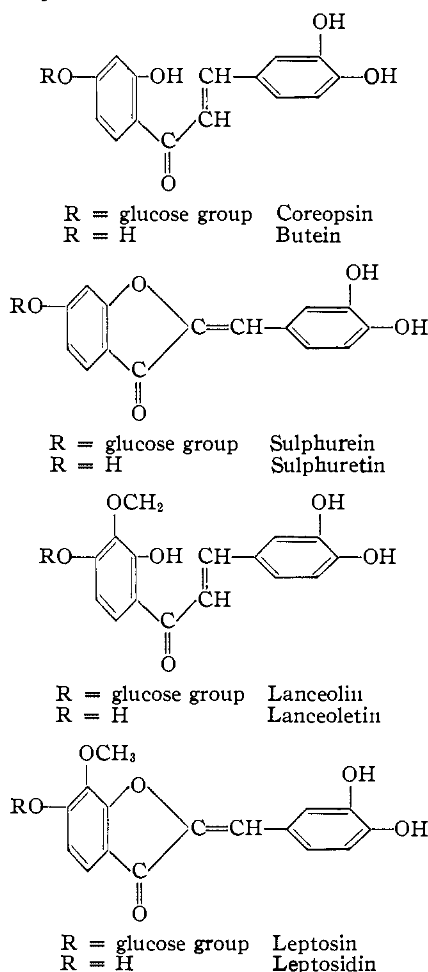
Although the nature and position of the sugar residue in coreopsin had not been previously determined, Geissman thought that the sugar probably is attached to either the 2′- or 4′-position of butein. This suggestion was based on the observation that the red color of a solution of coreopsin in alkali is similar to that shown by other chalcones hydroxylated in the 3,4-positions, and that the color is deeper than those of solutions of 4-hydroxy- or 4-hydroxy-3-methoxychalcone in alkali.

Using the procedure detailed in the experimental section below, the sugar of coreopsin has now been proved to be glucose attached to the 4′-position of butein.

Paper chromatographic studies of the ethanol extract of the flowers of *Cosmos sulphureus* indicated

the presence of butein, coreopsin and a new glycoside. After removal of the coreopsin, the mother liquor of this extract was further treated, as described in the experimental portion, to obtain crystals of the new glucoside, called "sulphurein." On hydrolysis with mineral acids, the sulphurein yielded one mole each of glucose and of sulphuretin, 3',4',6-trihydroxybenzalcoumaranone. Coreopsin was successfully converted to sulphurein, and the latter's constitution has been established as being the 6-glucoside of sulphuretin.

Paper chromatograms of the ethanol extract of the ray flowers of *Coreopsis lanceolata* and *C. saxicola* indicated leptosidin, a new chalcone glycoside, and leptosin to be present. The new chalcone glycoside has been named "lanceolin." The crystallization of lanceolin has not yet been achieved, but its crystalline acetate has been obtained. Lanecolin has been converted to leptosin, and may be considered to be 2',3,4-trihydroxy-3'-methoxy-4'-glucosidoxychalcone.



Experimental[2]

Isolation of Coreopsin.—Fresh ray flowers (1 kg.) of *Cosmos sulphureus* were boiled with 5, 4, 3 and again 3 l. of ethanol, resp., each time for 1 hr., and the ethanol extract was concentrated by distillation *in vacuo* to about 500 ml. After extracting 4–5 times with ether to remove free butein as well as ether-soluble carotenoids, the ethanol portion was further evaporated to about 200 ml. From this solution, coreopsin gradually precipitated in minute, yellow granules. These were filtered and washed with water (yield, about 3 g.). When reprecipitated from aqueous ethanol, a yellow crystalline powder was obtained, which sintered at 150° and melted at 190–195°. The substance showed all the characteristic properties of coreopsin as described by Geissman.[1] This glucoside could not be successfully crystallized.

Chromatograms of Extract of *C. sulphureus*.—Chromatograms were prepared, according to the directions of Bate-Smith[3] and of Gage,[4] using the three-component system *n*-butanol–acetic acid–water (4:1:5) as solvent and Toyo No. 50 filter paper at 28°. The ethanol extract of the flowers of *C. sulphureus*, before removal of butein and coreopsin, gave three spots (R_f: 0.92, 0.68, 0.55). The first two spots were identical with those of butein and coreopsin, resp. The third spot represents the new glycoside sulphurein. The spot of the new glycoside gave a purple coloration with aqueous sodium carbonate and exhibited a golden-yellow color in ultraviolet light. The benzalcoumaranone glucoside prepared from coreopsin heptaacetate gave the same R_f value (0.55) as did sulphurein.

After the ethanol extract was hydrolyzed with 3% HCl on a boiling water-bath for about 30 min. only one spot (R_f 0.92) was observed. Sulphuretin has an R_f value almost similar to that of butein.

Hydrolysis of Coreopsin.—The glycoside coreopsin (2 g.) was suspended in 100 ml. of water, 3 g. of concd. H_2SO_4 added, and the mixture boiled for 1 hr. On cooling, the aglycone gradually separated, at first in a resinous, and then in a yellow crystalline form. After standing overnight, the aglycone was filtered and recrystallized from 20% methanol (15 ml.). The resulting solid consisted of yellow needles, which melted at 212–214° and exhibited the properties of butein.

The filtrate separated from the aglycone above was neutralized with barium hydroxide, filtered, evaporated on a boiling water-bath to about 10 ml., and again filtered. Chromatograms were prepared, according to the directions of Partridge[5] and of Horrocks.[6] The three-component system *n*-butanol–acetic acid–water (4:1:1) was used as solvent and the benzidine solution as a developing agent. The filtrate gave only a single spot, and this corresponded to glucose (R_f 0.17). When the filtrate was heated on a boiling water-bath with phenylhydrazine hydrochloride (1 g.) and sodium acetate (1 g.), an osazone was formed after 7 min. After 30 min. of heating, the osazone was collected and recrystallized from 90% ethanol (12 ml.), yielding about 0.1 g. It decomposed at 204–206°, both alone and on admixture with an authentic specimen of glucosazone.

Coreopsin Heptaacetate.—To 0.2 g. of coreopsin in a small flask were added 1 g. of acetic anhydride and a drop of concd. sulfuric acid, and the mixture was stirred thoroughly with a glass rod. The reaction took place at once, while the glucoside dissolved to give an orange-red solution. Cold water was added, and the acetate was filtered, washed, and recrystallized from 20 ml. of methanol. After three recrystallizations, colorless needles were obtained. These melted at 171–173°, which checks well with the 171–172.5° reported by Geissman; yield 0.2 g. The addition of a few drops of 10% aqueous NaOH solution to the alcoholic solution yielded a red coloration in the cold.

Anal. Calcd. for $C_{35}H_{36}O_{17}$: C, 57.69; H, 4.98. Found: C, 57.66; H, 5.04.

Coreopsin Hexaacetate.—To 1 g. of coreopsin and 5 ml. of acetic anhydride in a small flask were added 2–3 drops of pyridine.[7] The reaction took place at once with development of heat, and a clear orange solution resulted, after 3 min. of stirring with a glass rod. After mixing with cold water, the acetate was extracted with about 0.75 l. of ether, and the solution was washed with sodium bicarbonate, and then evaporated. The residue was recrystallized from methanol (75 ml.). Pale yellow crystals were obtained. They developed a dark purple color with ferric chloride, and melted at 184–189°, showing the presence of a free hydroxyl group in the position ortho to the carbonyl.

(2) All melting points are not corrected.

(3) E. C. Bate-Smith, *Nature*, **161**, 835 (1948); E. C. Bate-Smith and R. G. Westall, *Biochem. Biophys. Acta*, **4**, 427 (1950).

(4) T. B. Gage, C. D. Douglass and S. H. Wender, *Anal. Chem.*, **23**, 1582 (1951).

(5) S. M. Partridge, *Nature*, **158**, 270 (1946).

(6) R. Horrocks, *ibid.*, **164**, 444 (1949).

(7) M. Shimokoriyama, *Bull. Chem. Soc. Japan*, **16**, 284 (1941).

Anal. Calcd. for $C_{33}H_{34}O_{16}$: C, 57.72; H, 4.99. Found: C, 57.86; H, 5.28.

Coreopsin Heptaacetate from the Hexaacetate.—Fifty ml. of the coreopsin hexaacetate was put in a small test-tube and acetylated as described above with acetic anhydride (0.5 ml.) and a droplet of H_2SO_4. When recrystallized from methanol, the acetate melted at 171–173°, both alone and on admixture with the heptaacetate mentioned above. From this fact, it has now been shown that the sugar residue is not attached to the 2′-position of the butein.

Preparation of a Benzalcoumaranone from Coreopsin Heptaacetate.—In order to obtain coreopsin in a pure crystalline state, an attempt was made to deacetylate its acetate under mild conditions, because the hydroxyl groups in the positions 3 and 4 are extremely sensitive to an alkaline medium. Accordingly, a solution of coreopsin heptaacetate (1 g.) in 50 ml. of methanol was mixed with sodium bicarbonate (0.1 g., 1 mol) and refluxed on a boiling water-bath. The mixture soon showed a red color. After heating 30 min. when about half of the bicarbonate had dissolved, 50 ml. of water was added, and the solution heated 30 additional minutes. On cooling, the mixture was allowed to stand for 2 days. When 1 drop of the solution was added to 1 N NaOH solution, a purplish color appeared. The reaction mixture was now concentrated under reduced pressure to about 10–15 ml. The orange-red solution was allowed to stand a week more and the coloration taking place on addition of aqueous alkali was quite purplish. The solution was then acidified with a few drops of dilute acetic acid, and exhaustively extracted with ethyl acetate. The acetate solution was evaporated, and the residue dissolved in 5 ml. of hot water, filtered, and let stand in the refrigerator. Orange-red crystals gradually precipitated; yield about 0.3 g., m.p. 185–200°.

When the above procedure was repeated with coreopsin, 50 ml. of methanol and 50 mg. of sodium bicarbonate ($^1/_2$ mol.), 0.6 g. of the same orange-red crystals were obtained, melting at 185–200°.

No trace of this compound was found when the procedure was performed in an atmosphere of nitrogen. Apparently unchanged coreopsin was recovered. The acceptor for hydrogen atoms, which must be freed from the chalcone in the course of the reaction involved, might be, therefore, atmospheric oxygen.

Estimation of water of crystallization: Found: (dried under reduced pressure over P_2O_5 at 110°) H_2O, 17.3, 17.2. $C_{21}H_{20}O_{10}\cdot 5H_2O$ requires 17.2% H_2O.

Anal. Calcd. for $C_{21}H_{20}O_{10}$: C, 58.33; H, 4.66. Found: C, 58.06; H, 4.58.

From the analytical data and color reactions, this compound appears to be a benzalcoumaranone glucoside. It gave reactions quite similar to those of leptosin.

Hydrolysis of Coreopsin Heptaacetate.—The acetate (1.2428 g.) was dissolved in hot methanol (50 ml.), 6% hydrochloric acid (50 ml.) added, and the mixture was boiled 36 hours on a boiling water-bath. The solution was evaporated under reduced pressure, water (100 ml.) was added, and the aqueous solution was extracted 8 times with ether. The ethereal solution was evaporated, and the red resinous residue, consisting probably of butein and butin, was dried in a small flask; yield 0.523 g. (found, 42.0%; calcd., 37.4%).

The above liquor, removed from the aglycone, was concentrated and transferred into a volumetric flask and filled up with water to 50 ml. With 10 ml. of this solution the sugar was quantitatively determined according to the method of Bertrand. It was found that the reducing power of each 10 ml. of the solution was equal to 13.0 ml. of $^1/_{10}$ N $KMnO_4$. Found: 17.3% (215 mg. glucose); calcd., 24.7% (308 mg. glucose).

The amount of glucose thus estimated is too small, probably due to its degradation by long heating. It may be, however, practically enough to conclude that coreopsin is composed of 1 mole each of butein and glucose.

Sulphurein.—The mother liquor of the extract from fresh ray flowers (1 kg.), after removal of coreopsin, showed a purplish coloration with aqueous alkali, and was exhaustively extracted with ethyl acetate (10 extractions were necessary). The combined acetate solution was evaporated under reduced pressure and the residue was dissolved in hot water (50 ml.). After filtering, this solution was again extracted with ethyl acetate. The residue of the extract was dissolved in hot water (15 ml.) and the solution was allowed to stand. Orange red crystals gradually separated; yield 6.0 g., m.p. 185–200°. The glucoside possesses the same properties as the glucoside prepared from coreopsin. When the orange-red crystals were dried over concd. sulfuric acid or phosphorus pentoxide, the color turned to a dull yellow, owing to dehydration. The color returned, however, to orange-red in open air.

Hydrolysis of Sulphurein.—Dried sulphurein (0.7461 g.) was dissolved in hot water (20 ml.), 20% hydrochloric acid (2 cc.) added, and the whole was heated on a boiling water-bath for 45 min. On cooling, crystals of the aglycone separated. After neutralization with sodium bicarbonate and acidification with a few drops of dilute acetic acid, the mixture was extracted 5–6 times with a mixture of ether and ethyl acetate (1:1). The extract was evaporated, and the residue was placed in a small flask, dried and weighed; yield of a resinous red-brown product mixed with crystals, 0.486 g. Found: 65.2%; calcd. for $C_{21}H_{20}O_{10}$, 62.5%.

From the mother liquor, freed from the aglycone sulphuretin, glucosazone was obtained in crystals of m.p. 204–207°, both alone and on admixture with authentic specimen. No other sugar osazone was obtained.

The aglycone sulphuretin was recrystallized from dilute alcohol and obtained in yellow crystals; m.p. 280–285°. When added to an aqueous solution of sodium hydroxide, a purplish-red coloration appeared, which resembled that of leptosidin.

Anal. Calcd. for $C_{15}H_{10}O_5$: C, 66.66; H, 3.73. Found: C, 66.78; H, 3.86.

Sulphuretin Triacetate.—Sulphuretin (0.2 g.) was acetylated with 1 cc. of acetic anhydride and a droplet of concd. sulfuric acid, in the usual way. The triacetate crystallized from methanol in pale yellow needles of m.p. 191–194°.

Anal. Calcd. for $C_{21}H_{16}O_8$: C, 63.64; H, 4.07. Found: C, 63.91; H, 4.33.

Isolation of Leptosin.—A lot of fresh ray flowers (500 g.), this time that of *Coreopsis lanceolata*, was extracted with boiling ethanol (1.5 l.) for 1 hr. The extraction was repeated three additional times with fresh ethanol, and the combined alcoholic solution was evaporated under reduced pressure to about 200 ml. This was shaken several times with ether in order to remove carotenoids and other ether-soluble substances, and the aqueous solution was again evaporated to about 100 ml. On standing red-orange crystals gradually separated. After a week or more, the crystals were collected (about 2 g.), and recrystallized from 100 times their weight of 50% methanol or ethanol. These orange-red needles of leptosin turned yellow at 125–130°, became orange-red at 160°, again yellowish at 230° and melted finally at 230–235°. Crystals of leptosin, freshly separated from dilute ethanol or methanol and showing an orange-red color, changed into yellow, when treated with cold methanol, while the crystals took a minute, short lancet form. Leptosin showed a purple coloration with aqueous alkali, which changed on standing to reddish-brown, and its ethanolic solution gave a brownish coloration with ferric chloride, and a deep orange-red coloration on adding some magnesium acetate. The latter color reaction was first described by Shibata[8] for the detection of polyhydroxyanthraquinones. The glucoside showed further halochromic color change into a deep red with concd. hydrochloric or sulfuric acid. The substance shared all properties with the leptosin described by Geissman.[1] The ray flowers of *C. saxicola* gave almost the same result, namely, 200 g. of fresh material yielded 0.85 g. of leptosin.

Estimation of water of crystallization. Found: (dried under reduced pressure over P_2O_5 at 110°) H_2O, 10.9, 10.6. $C_{22}H_{22}O_{11}\cdot 3H_2O$ requires 10.4% H_2O.

Anal. Calcd. for $C_{22}H_{22}O_{11}\cdot 3H_2O$: C, 51.16; H, 5.46. Found: C, 51.47; H, 5.77.

Chromatograms of Extracts of *C. lanceolata* and *C. saxicola*.—Chromatograms of the ethanol extract of the ray flowers of *C. lanceolata* and *C. saxicola* were prepared as described above for *C. sulphureus*. In these cases, three spots were easily detected (R_f: 0.88, 0.67 and 0.53, resp.). These correspond to leptosidin, a new chalcone glucoside and leptosin. The new chalcone glycoside was named "lanceolin," and was later obtained as its crystalline acetate.

(8) S. Shibata, *J. pharm. Soc. Japan*, **61**, 320 (1941).

Hydrolysis of Leptosin.—Dried leptosin glucoside (0.9828 g.) was suspended in hot water (50 ml.), concd. sulfuric acid (1.5 g.) added, and the mixture was heated for 3 hr. on a boiling water-bath. On standing overnight, the deposited aglycone was filtered, washed and dried; yield 0.5959 g. (found 60.6%, calcd. 65.0%).

From the above filtrate, glucosazone (m.p. 204–206°) was obtained by the usual method.

Leptosin Hexaacetate.—Leptosin (0.5 g.) was acetylated with acetic anhydride (5 ml.) and one drop of concd. sulfuric acid. The reaction mixture showed a momentary purplish-brown coloration. Soon pale yellow crystals appeared in the mixture and the whole solidified. After adding water, the crystals were filtered off and recrystallized from 500 times their weight of ethanol. Pale yellow needles resulted, which melted at 232–234° as described by Geissman.

Anal. Calcd. for $C_{34}H_{34}O_{17}$: C, 57.14; H, 4.80. Found: C, 57.59; H, 5.09.

Leptosidin and Its Triacetate.—The aglycone from leptosin was recrystallized from dilute alcohol and obtained in yellow needles, which melted at 236–240°.

Anal. Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 62.88; H, 4.12.

Leptosidin (0.05 g.) was treated in a small test-tube with acetic anhydride (0.5 ml.) and pyridine (2 drops) under stirring with a glass rod. In a few minutes, the liquid warmed itself, the crystals dissolved, and pale yellow needles separated in their place. Cold water was then added, and the crystals were filtered, washed, and recrystallized from 100 times their weight of 90% ethanol. Pale yellow needles resulted, which melted at 164–166° as described by Geissman.

Anal. Calcd. for $C_{22}H_{18}O_9$: C, 61.97; H, 4.26. Found: C, 61.83; H, 4.52.

Isolation of Lanceolin Acetate.—The mother liquor of the extract from fresh ray flowers (500 g.) of *Coreopsis lanceolata*, with leptosin removed, was exhaustively extracted with ethyl acetate by shaking, and the solvent was evaporated under reduced pressure. The residue was treated with acetic anhydride (50 ml.) and pyridine (2 ml.), and the mixture was heated for 30 min. This treatment was not sufficient for complete acetylation, since a small portion of the reaction mixture showed a positive ferric chloride reaction. So the solvent was evaporated *in vacuo* and the same procedure was repeated. On cooling, the mixture was triturated with cold water until the product solidified, and this solid mass was filtered, washed thoroughly with water, dissolved in methanol (100 ml.) and allowed to stand. Almost colorless needles separated from the solution (about 1.2 g.). These were recrystallized from 100–150 times their weight of methanol. Colorless needles resulted, which melted at 215–220°. The ethanolic solution gave a red coloration with a few drops of 10% sodium hydroxide solution in the cold, contrary to that of leptosin acetate, which showed a purple coloration. By the same procedure 200 g. of fresh ray flowers of *Coreopsis saxicola* afforded about 0.5 g. of acetate.

Anal. Calcd. for $C_{36}H_{38}O_{18}$: C, 56.99; H, 5.05. Found: C, 56.52; H, 4.50.

Leptosin Obtained from Lanceolin Heptaacetate.—Lanceolin acetate (0.6 g.) was dissolved in hot methanol (60 ml.), sodium bicarbonate (60 mg., 1 mol) was added, and the mixture was refluxed for 15 min. on a boiling water-bath. To the resulting red solution, water (30 ml.) was added and the whole was heated 30 min. more. After standing a day, the mixture was concentrated to 15 ml. under reduced pressure. One drop of the solution gave a purplish coloration with 1 N sodium hydroxide solution, characteristic of the formed benzalcoumaranone. In a few days, reddish-orange crystals of leptosin gradually separated. After a week of standing, the crystals were filtered by suction and washed with water; yield 0.35 g. When recrystallized from 80% methanol (60 ml.), the crystals melted at 230–235°, both alone and admixed with the authentic specimen of leptosin.

Leptosin Acetate Prepared from Lanceolin Acetate.—Leptosin (0.1 g.) thus prepared from lanceolin acetate was acetylated with acetic anhydride (2 ml.) and pyridine (2–3 drops) by heating for 10 min. on a boiling water-bath. The crystals soon dissolved, and pale yellow crystals of acetate separated. After addition of some cold water, the crystals were collected, washed with water and recrystallized from 500 times their weight of methanol. Pale yellow needles were obtained, which melted at 232–234°, both alone and admixed with the authentic specimen of leptosin acetate.

Hydrolysis of Lanceolin Acetate.—Lanceolin acetate (1.135 g.) was suspended in 50% alcohol (30 ml.), concd. sulfuric acid (1 g.) was added, and the mixture was refluxed on a boiling water-bath. After 30 min. heating, the crystals dissolved and a clear orange colored solution resulted. The mixture was heated 30 min. more, neutralized after cooling with sodium bicarbonate, and then evaporated. Water was added, and the solution was extracted with a mixture of ether and ethyl acetate (1:1). The extract was evaporated to dryness and weighed; yield 0.492 (found, 43.4%; calcd., 39.8%).

The above mother liquor, freed from the aglycone, was evaporated, the residue was added with 90% ethanol, and the solution was filtered and evaporated again. From the aqueous solution of the residue, solely glucosazone (m.p. 204–206°) was obtained (40 mg.).

Lanceoletin and Its Acetate.—The above mentioned aglycone is probably a mixture of chalcone and flavanone, and because of the difficulty to fractionate and purify them, the mixture was vigorously acetylated to give a peracetate of lanceoletin.

The mixture of lanceoletin and the corresponding flavanone derivative was heated for an hour on a boiling water-bath with sodium acetate (0.5 g.) and acetic anhydride (20 cc.). The reaction mixture was treated with cold water, and the precipitate was filtered and recrystallized from methanol. Pale yellow prisms melting at 162–166° were obtained. Geissman isolated from the ray flowers of *Coreopsis grandiflora* a flavanone, 8-methoxybutin, and prepared its tetraacetate, which melted at 106–107°. The chalcone lanceoletin, is now to be considered undoubtedly to possess the constitution of 8-methoxybutein, but the scarcity of available amount does not permit us to elucidate its structure.

Discussion

Our preliminary survey of yellow flowers of some composite plants, such as *Dahlia*, *Bidens* (especially *B. laevis* (L.) Britton, Stern et Poggendorf), and *Coreopsis tinctoria* Nuttall, which might contain so-called anthochlor pigments, has revealed the presence of two distinct glycosidic coloring matters, chalcone and benzalcoumaranone. On the chromatogram, two spots corresponding to these were usually obtained, the upper one had an R_f value about 0.65 with the above-mentioned solvent system, and the lower one had an R_f value of approximately 0.50 with the same solvent. The identification of these two sorts of pigments remains to be investigated.

The co-existence in the ray flowers of *Coreopsis* and *Cosmos* of a benzalcoumaranone glycoside and a chalcone glycoside of corresponding structure was, though in a few instances, thus established, a fact which may offer an interesting example in view of the biogenetical relationship of closely related substances. This discovery probably makes us expect that similar circumstances will be found in other composite plants of the sub-tribe *Coreopsidinae*, to which the genera *Coreopsis*, *Cosmos*, *Bidens*, etc., belong.

Although conversion of chalcone glycoside into the corresponding benzalcoumaranone glycoside by means of a comparatively simple procedure has been accomplished as described above, little is known at present with regard to its mechanism.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Pteridines. X. A New Approach to the Synthesis of Pteridines[1]

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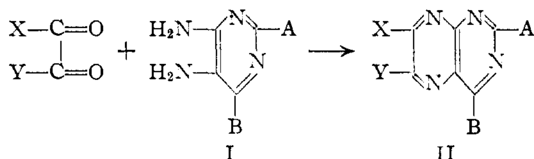
A new approach to the synthesis of pteridines is described. The method involves (a) the preliminary synthesis of a 2,4-(1H,3H)pteridinedione (lumazine) by the conventional method; (b) aminolytic cleavage of the pyrimidine portion of the lumazine to give a 3-amino-N-substituted pyrazinamide; and (c) ring reclosure of this pyrazinamide to the desired pteridine. This approach retains the advantages of the conventional synthetic procedures with regard to the ease of building up the pyrazine ring and permits a much wider variation in the structure of the pyrimidine ring. This paper records some model experiments illustrating step (c), the cyclization to pteridines of the pyrazinamides formed by cleavage of lumazines with primary amines.

The conventional methods for the synthesis of the pteridine ring system employ a condensation of a 4,5-diaminopyrimidine (I) with an α,β-dicarbonyl compound, an α-halocarbonyl compound, an α-keto alcohol or derivatives of these compounds.[2] Almost all of the known synthetic pteridines have been prepared by one of these routes. Although these methods allow considerable variation in the groups X and Y on the pyra-



zine ring of the resulting pteridine (II), considerably less variation has been possible in the substituents on the pyrimidine ring because of the limitations encountered in the synthesis of the requisite 4,5-diaminopyrimidines (I). Thus, substituents A and B have been restricted principally to amino or substituted amino, hydroxy or mercapto groups or to hydrogen. Only a few examples are known of pteridines substituted by alkyl groups in positions 2 and 4[3-8] or by alkyl groups on the ring nitrogen atoms in the pyrimidine ring.[9]

We wish to describe preliminary results on a new approach to the synthesis of pteridines which retains the advantages of the conventional methods with regard to the ease of placing the substituents X and Y, but whose special feature is the varied manner in which the pyrimidine ring may be constructed. In brief, the method involves (a) the preliminary synthesis of a 2,4(1H,3H)pteridinedione (lumazine) by the conventional method; (b) aminolytic cleavage of the pyrimidine portion of the lumazine to give a 3-amino-N-substituted pyrazinamide; and (c) ring reclosure of this pyrazinamide to the desired pteridine. Step (a) has been reviewed extensively,[2] and step (b) has been discussed recently with respect to the action of alkylamines on 6,7-diphenyl-2,4(1H,3H)pteridinedione (6,7-diphenyllumazine) (III) to give 3-amino-N-substituted-5,6-diphenylpyrazinamides (IV, V).[10] The present paper records some model experiments illustrating step (c), the cyclization to pteridines of the pyrazinamides formed by cleavage of lumazines with primary amines. Although all of the following cyclization experiments involve 5,6-diphenylpyrazinamides derived from 6,7-diphenyllumazine (III), it will be shown in later communications that step (c) is independent of the nature of the 5- and 6-substituents, and that the synthetic method comprising steps (a), (b) and (c) is entirely general.

A few examples are known of ring closure of pyrazinamides to pteridines. Gabriel and Sonn[11] prepared 2,4(1H,3H)pteridinedione (lumazine) from pyrazine-2,3-dicarboxamide in 40% yield by use of the Hofmann reaction. Albert, *et al.*,[12] cyclized 3-aminopyrazinamide to 4(3H)pteridinone with ethyl orthoformate and acetic anhydride and also with formic acid and acetic anhydride; by the same methods, 3-aminothiopyrazinamide was cyclized to 4(3H)pteridinethione. From this Laboratory, it has been reported that the action of formic acid and acetic anhydride on 3-amino-N-benzyl-

(1) Taken in part from theses presented by John A. Carbon and Dale R. Hoff to the University of Illinois in partial fulfillment of the degree of Bachelor of Science in Chemistry.

(2) For extensive reviews, see (a) M. Gates, *Chem. Revs.*, **41**, 63 (1947); (b) J. A. Elvidge, *Ann. Rep. Prog. Chem. (London)*, **45**, 226 (1948); (c) H. N. Rydon, *ibid.*, **47**, 241 (1950); (d) A. Albert, *Rev. Pure App. Chem.*, **1**, 51 (1951); (e) A. Albert, *Quart. Revs.*, **6**, 197 (1952).

(3) H. Andersag and K. Westphal, *Ber.*, **70B**, 2035 (1937).

(4) W. C. J. Ross, *J. Chem. Soc.*, 1128 (1948).

(5) G. R. Ramage, British Patent, 619,915 (1949); *C. A.*, **43**, 9087 (1949).

(6) M. Polonovski and H. Jerome, *Compt. rend.*, **230**, 392 (1950); M. Polonovski, M. Pesson and A. Puister, *ibid.*, **230**, 2205 (1950).

(7) M. Pesson, *Bull. soc. chim. France*, **15**, Ser. 5, 963 (1948).

(8) H. S. Forrest, R. Hull, H. J. Rodda and A. R. Todd, *J. Chem. Soc.*, 3 (1951).

(9) (a) F. Sachs and G. Meyerheim, *Ber.*, **41**, 3957 (1908); (b) H. von Euler, K. M. Brandt and G. Neumüller, *Biochem. Z.*, **281**, 206 (1935); (c) R. Kuhn and A. H. Cook, *Ber.*, **70B**, 761 (1937); (d) M. Pesson, *Bull. soc. chim. France*, **15**, Ser. 5, 963 (1948); (e) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **73**, 2864 (1948).

(10) E. C. Taylor, Jr., *ibid.*, **74**, 1651 (1952).

(11) S. Gabriel and A. Sonn, *Ber.*, **40**, 4850 (1907).

(12) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 474 (1951). NOTE ADDED IN PROOF.—A more recent article by these authors, *ibid.*, 4219 (1952), describes the cyclization of 3-amino-N-methylpyrazinamide to 3-methyl-4(3H)-pteridinone with formic acid and acetic anhydride.