ribonucleotides were separated by ion-exchange chromatography.[11] The polysaccharide impurity was not adsorbed, and was washed off the column before the first nucleotide was eluted. Cytidylic acid, in a thin layer on a planchet, showed 750 counts/min./micromole. Adenylic and guanylic acids were separately hydrolyzed with hydrochloric acid, the purines isolated on cation-exchange columns, precipitated as copper salts, and converted to barium carbonate for counting. Cytidylic and uridylic acids were hydrolyzed with perchloric acid,[12] the free bases isolated on cation and anion exchange resins, respectively, precipitated as silver salts, and converted to barium carbonate. (The uracil is believed to have contained some carbohydrate impurity.) Approximately 0.25 mg. of uracil was diluted with 5 mg. of non-labeled uracil and degraded [2] The results shown below indicate incorporation of the carbamyl carbon of citrulline into carbon 2 of pyrimidines.[13]

		Counts/min./mg. C[a]
Citrulline-carbamyl-C^{14}		66,000
Carbon dioxide, 2nd day		29
Carbon dioxide, 3rd day		16
Carbon dioxide, 4th day		11
Protein		20
Nucleic acid	Guanine	9
	Adenine	8
	Cytosine	800
	Uracil	670
	CO_2[b]	2
	Oxalate	0
	Urea (carbon 2)	185

[a] $BaCO_3$ plates, counted in a gas-flow counter, and corrected to infinite thickness. [b] Uracil degradation products, not corrected for approximately 20-fold dilution.

(11) W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950).

(12) A. Marshak and H. J. Vogel, *J. Biol. Chem.*, **189**, 597 (1951).

(13) While this manuscript was in preparation, the abstract by M. P. Schulman and S. J. Badger appeared (*Fed. Proc.*, **13**, 292) showing a similar incorporation in pigeons.

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SYNTHESIS OF THE TRIS-(DIMETHYLGLYOXIMO)-COBALTATE(III)

Sir:

Since the work of Tschugaeff,[1] a number of dimethylglyoximo-cobalt(III) complexes have been prepared. However, the complex compound having the internal tri-salt structure, *i.e.*, the complex compound which is formed by the coördination of three ions[2] of dimethylglyoxime about a cobalt atom, has never been described. Thus it has been a definite opinion that dimethylglyoxime cannot fill more than four coördination positions even when introduced into the six-coördinate complex.[3,4,5]

(1) L. Tschugaeff, *Z. anorg. Chem.*, **46**, 144 (1905); *Ber.*, **39**, 2692 (1906); **40**, 3498 (1907); **41**, 2226 (1908).

(2) The ion of dimethylglyoxime (DMG) = $CH_3C(NO)C(NOH)CH_3$.

(3) F. G. Mann, *J. Chem. Soc.*, 412 (1933).

(4) H. J. Emeleus and J. S. Anderson, "Modern Aspects of Inorganic Chemistry," 2nd ed., George Routledge and Sons, Ltd., London, 1952, p. 125.

(5) L. Cambi and C. Coriselli, *Gazz. chim. ital.*, **66**, 91–96 (1936).

This concept is no longer valid since the present authors have succeeded in preparing the tris-dimethylglyoximo-cobaltate(III).

Six grams of dimethylglyoxime was dissolved completely in 60 ml. of hot water containing 6 g. of potassium hydroxide. After the solution was cooled to 40–50°, 5 g. of crystalline cobaltous nitrate hexahydrate was added. The mixture was shaken vigorously and thoroughly, and to this 12 ml. of 50% acetic acid was added. Then the air was bubbled vigorously through the reaction mixture for about three hours. Beautiful orange-yellow acicular crystals gradually were deposited. The mixture was allowed to stand for several hours, and then filtered by suction. The crude substance was recrystallized from water containing a small amount of acetic acid. Four grams of pure substance was obtained.

It is quite stable in the solid state and hardly decomposes below 180°. The crystal appears orange-yellow or brownish-yellow. It is almost insoluble in benzene and acetone, and is soluble in water, alcohol, chloroform and dioxane. It is soluble in dilute acetic acid.

In place of the potassium hydroxide and cobaltous nitrate in the above described procedure, sodium hydroxide and cobaltous chloride, respectively, may be used. Analyses were: Calcd. for $[Co(DMG)_3]\cdot 2.5H_2O$: Co, 13.12; C, 32.08; H, 5.83; N, 18.71; H_2O, 10.02. Found: Co, 12.90; C, 32.51; H, 5.98; N, 18.80; H_2O, 9.75.

Furthermore, it will also be concluded that three ions of dimethylglyoxime coördinate about a cobalt atom as the chelate ligands, similar to the ethylenediamine in the tris-(ethylenediamine)-cobalt(III) complex ion. If this proves to be the case the complex molecule must be optically active. Since the compound, however, could not be resolved by ordinary methods, we adopted the method of asymmetric adsorption by quartz.[6] From the results of this experiment a poor but definite optical activity was confirmed, supporting the above conclusion.

(6) R. Tsuchida, M. Kobayashi and A. Nakamura, *J. Chem. Soc. Japan*, **56**, 1339 (1935); *Bull. Chem. Soc. Japan*, **11**, 38 (1936).

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RECEIVED APRIL 28, 1954

THE ISOLATION OF PODOPHYLLOTOXIN GLUCOSIDE

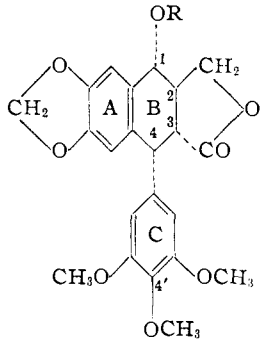
Sir:

It has hitherto been found possible to isolate several crystalline compounds from the resin fraction of certain species of *Podophyllum* (*Berberidaceae* family) which are characterized by a noteworthy biological activity. The most important and the one occurring in the greatest quantity is podophyllotoxin.[1] This is present in the American species *Podophyllum peltatum* L. and in the Indian species, *P. emodi* Wall. The Indian plant also contains a compound which has one methyl

(1) V. Podwyssotzki, (a) *Arch. Exp. Path.*, **13**, 29 (1880); (b) *Ber.*, **15**, 377 (1882).

group less, 4′-demethylpodophyllotoxin[2]; further active substances, α-peltatin[3] and β-peltatin[3c,4] have been isolated from the American plant. These well-defined compounds are of particular interest in view of their damaging effect on experimental tumors.[5]

Podophyllotoxin and the above-mentioned compounds derived from species of *Podophyllum* are closely related chemically; Hartwell and Schrecker[6] have proposed the formula Ia for podophyllotoxin.



Ia, R = H
Ib, R = glucose residue

Of the isomers possible in view of the four asymmetric C atoms in ring B, the natural substance very probably has a *trans* (1:2), *trans* (2:3), *cis* (3:4) configuration.[7]

Podophyllotoxin yields by treatment with alkali the biologically inactive picropodophyllin[1a] which differs only with regard to the steric arrangement of the substituents in the saturated ring B (*cis* (2:3), *trans* (3:4)). The β-D-glucoside of picropodophyllin, which has no effect on tumors, could be isolated from the Indian drug.[2b]

Podophyllotoxin and the active compounds with a similar structure are not readily soluble in water. This is a disadvantage for biological investigations and we have therefore studied the *Podophyllum* drugs to determine the presence of a glycoside of an active substance. We have succeeded in obtaining, in a pure and homogeneous form, the β-D-glucoside of podophyllotoxin from the methanolic extract of Indian *Podophyllum emodi* Wall. after separating the resin fraction and the tannins. The yield from commercial supplies of the drug amounted to approximately 0.5–1%.

Podophyllotoxin-β-D-glucoside (Ib) is a white amorphous powder which exhibits no tendency to crystallize. The glucoside melts at 149–152°, and and has the specific rotation $[\alpha]^{20}D$ −65° (*c* 0.5) in water, $[\alpha]^{20}D$ −75° (*c* 0.6) in methanol and $[\alpha]^{20}D$ −117° (*c* 0.67) in pyridine. Analysis gave the formula $C_{28}H_{32}O_{13}\cdot{}^1/_2\ H_2O$ (Calcd.: C, 57.43; H, 5.68; O, 36.89; OCH_3, 15.90. Found: C, 57.30; H, 5.60; O, 36.60; OCH_3, 15.92). The ultraviolet absorption spectrum is practically identical with that of podophyllotoxin[8] (λ_{max} = 291 mμ, log ε = 3.62). The glucoside is very readily soluble in alcohols, acetone and ethyl acetate and is readily soluble in water. A 2% aqueous solution is stable.

The glucoside is rapidly and completely split by β-glucosidase (emulsin) to produce podophyllotoxin which is characterized by its properties (crystallizes in needles m.p. 114–117°; from benzene–petroleum ether, $[\alpha]^{20}D$ −132°, *c* 0.6, chloroform) and by those of its acetyl derivative (thin prisms from absolute alcohol with m.p. 210–211°; $[\alpha]^{20}D$ −134°, *c* 0.6, chloroform). The sugar was identified as D-glucose. The ready cleavage by emulsin suggests that the glucoside has the structure 1-O(-β-D-glucopyranosyl)-podophyllotoxin.

Treatment of the glucoside with potassium hydroxide rapidly and quantitatively produces the isomeric, easily crystallizing but not readily soluble picropodophyllin glucoside, which is characterized by having two m.ps. at 235–236°/252–254° and an optical rotation of $[\alpha]^{20}D$ −10.5° (*c* 0.65, pyridine).

The tetra-acetyl derivative ($C_{36}H_{40}O_{17}$, calcd.: C, 58.06; H, 5.41; O, 36.53. Found: C, 58.04; H, 5.51; O, 36.36%) is also suitable for characterizing the podophyllotoxin glucoside. It crystallizes from five parts of methanol in small prisms melting at 134–135°, with an optical rotation of $[\alpha]^{20}D$ −90.8° (*c* 0.63, chloroform). Even mild treatment with alkali to split off the acetyl groups yields picropodophyllin glucoside, the physiologically inactive product of isomerization of the natural substance.

These observations confirm that the compound newly isolated from *P. emodi* Wall. is the glucoside of the principal active substance, podophyllotoxin. This glucoside is, in view of biological studies, particularly characterized by its solubility in water and its ready cleavage by β-glucosidase which is present in most cells. Details of the preparation and properties will be published in *Helv. Chim. Acta*.

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(2) (a) M. V. Nadkarni, P. B. Maury and J. L. Hartwell, THIS JOURNAL, **74**, 280 (1952); (b) M. V. Nadkarni, J. L. Hartwell, P. B. Maury and J. Leiter, *ibid.*, **75**, 1308 (1953).

(3) (a) J. L. Hartwell, *ibid.*, **69**, 2918 (1947); (b) J. L. Hartwell and W. E. Detty, *ibid.*, **72**, 246 (1950); (c) J. L. Hartwell, A. W. Schrecker and G. Y. Greenberg, *ibid.*, **74**, 6285 (1952).

(4) J. L. Hartwell and W. E. Detty, *ibid.*, **70**, 2833 (1948).

(5) See review by M. G. Kelly and J. L. Hartwell, *J. Nat. Cancer Inst.*, **14**, 967 (1954).

(6) J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **73**, 2909 (1951). A formula with a further CH_3 group and a six-membered unsaturated lactone ring has been recently proposed by J. Press and R. Brun, *Helv. Chim. Acta*, **37**, 190 (1954).

(7) A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **75**, 5916 (1953).

(8) Ref. 3b, p. 251.