new species of the genus *Voacanga*, *Voacanga dregei* E.M. We wish to report the isolation of a new alkaloid, dregamine, from the bark of this tree[2].

Dregamine is a representative of 2-acyl indole alkaloids[3]; it was isolated[4] by chromatography of alkaloids obtained by benzene extraction of the bark of the trunk put at our disposal through the cooperation of Mr. J. L. SIDEY (Pietermaritzburg, Natal, S. Africa), who also established the botanical authenticity of the plant material with herbarium specimen (fruit and leaves). Elution with benzene-chloroform mixture (3:1) gave the crude base which crystallized from methanol in long prisms; m.p. 106–109°C, resolidified, then m.p. 186–205°C (dec.); $[\alpha]_D^{26} = -93{\cdot}1°C$ ($CHCl_3$, C = 1).

Calculated for $C_{21}H_{28}O_3N_2$: C 70·76; H 7·92; N 7·86. Found: C 70·57; H 7·47; N 7·42. The hydrochloride was prepared in the conventional manner and recrystallized from methanol-ether, m. p. 249–250°C (dec.). Calculated for $C_{21}H_{28}O_3N_2 \cdot HCl$: C 64·19; H 7·74; N 7·13; Cl 9·02; OCH_3 (1) 7·90; (N)-CH_3 (1) 3·83. Found: C 64·49; H 7·62; N 7·00; Cl 8·98; OCH_3 8·12; (N)-CH_3 3·84.

The ultraviolet spectrum of dregamine is characterized by the following bands: λ_{max}^{EtOH} 239 mμ, $a_M = 15{,}200$; 316 mμ, $a_M = 18{,}600$ and very similar to that of 1-keto-1,2,3,4-tetrahydrocarbazole[3]. In addition to the 2-acyl indole moiety (major band at 6·05 μ)[1], the infrared spectrum indicated the presence of a carbomethoxyl moiety with absorption at 5·78 μ and 8·03 μ[5].

Other botanically related genera are currently under investigation in these laboratories.

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N. NEUSS and NANCY J. CONE

Lilly Research Laboratories, Indianapolis (Indiana), July 6, 1959.

Zusammenfassung

Dregamine, ein neues Alkaloid aus der Apocynaceae *Voacanga dregei* E. M. wurde isoliert und charakterisiert. Diese Verbindung stellt einen neuen Vertreter der Klasse der 2-Acyl-indole dar.

[2] After the completion of this work, SCHULER, VERBEEK, and WARREN, J. chem. Soc. *1958*, 4776, have reported the isolation of the known alkaloids vobtusine and voacangine from the bark of *Voacanga dregei* collected in the South Coast, Natal, S. Africa. We were unable to find these alkaloids in our plant material.

[3] M. GORMAN, N. NEUSS, and N. J. CONE, Amer. chem. Soc. nat. Meeting, San Francisco, Calif., April 1958. The spectral data reported for voacafrine and voacafricine [K. V. RAO, J. org. Chem. *23*, 1455 (1958)] are also indicative of the presence of a 2-acyl indole moiety in these two alkaloids from *Voacanga africana*.

[4] Small amounts of this alkaloid were also isolated from *Ervatamia coronaria*[3].

[5] Subsequent to the preparation of this manuscript, RENNER has described the isolation of vobasine and voacryptine from *V. africana*. The former alkaloid appears to be another representative of 2-acyl indoles[3] [U. RENNER, Exper. *15*, 185 (1959)].

A Simple Synthesis of Nicotinic Aldehyde

Practically all known routes leading to nicotinic aldehyde involve the reduction of various nicotinic acid derivatives. We have now found a simple and advantageous method for the synthesis of this valuable compound based on crotonaldehyde.

Recently we have shown[1,2] that the Vilsmeier-Haack reaction may be extended to the aliphatic series, in particular to carbonyl compounds. By this reaction, we have prepared a large number of β-dicarbonyl derivatives, such as the β-dialdehydes, β-chlorovinylaldehydes and some novel types of polyformyl derivatives. Most of these compounds had previously been difficult of access.

$CH_2=CH-CH=CH-N(CH_3)_2$ (I)

$CH_3-CH(N(CH_2)_2)-CH=CH-N(CH_3)_2$ (II)

$(CH_3)_2N-CH=C(CH=O)-CH=CH-CH=O$ (III)

$[(CH_3)_2N-CH=CH-CH=CH-CH-N(CH_3)_2]\ X$ (IV)

We have now applied the reagent prepared from dimethylformamide and phosgene to compounds I and II, which are readily available from crotonaldehyde in a single step[3,4]. This reaction led to the trialdehyde derivative III in about 60% yield. This derivative is even more simply obtained by formylation of the well known quaternary salt IV which is also believed to be an intermediate in the formylation of I and II. The trialdehyde derivative III very readily passes into nicotinic aldehyde in excellent yield, e.g. merely on heating with aqueous NH_4Cl.

Nicotinic aldehyde is thus available from crotonaldehyde in a simple three-step process in about 40% overall yield.

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Department of Organic Synthesis, Institute of Chemistry, Czechoslovak Academy of Science, Prague, July 17, 1959.

Zusammenfassung

Nikotinaldehyd wurde durch ein dreistufiges Verfahren aus Krotonaldehyd in einer Gesamtausbeute von 40% erhalten.

[1] Z. ARNOLD and F. ŠORM, Coll. Czechoslov. chem. Comm. *23*, 452 (1958). – Z. ARNOLD and J. ŽEMLICKA, Coll. Czechoslov. chem. Comm. *24*, 786, 2378, 2385 (1959); *24*, in press.

[2] Z. ARNOLD and J. ŽEMLICKA, Proc. chem. Soc. *1958*, 227.

[3] C. MANNICH, K. HANDKE, and K. ROTH, Chem. Ber. *69*, 2112 (1936).

[4] W. LANGENBECK and L. WESCHKY, DRP 715544, Chem. Zbl. *1942*, 2821.

Demonstration of Poliomyelitis Virus in Homogenates and Ureadesoxycholate Lysates of Cells Exhibiting or Lacking Cytopathogenicity

A method for dissolution of tissue cultures (TC) with Ureadesoxycholate (UDC) was described previously[1]. Ap-

[1] E. KOVÁCS, Naturwissenschaften *45*, 339 (1958); Arch. Biochem. Biophys. *76*, 546 (1958).

Table. Infectivity of TC Homogenates or lysates

Total of Tubecultures pooled for assays	Type of fraction assayed	No. of cultures inoculated: time and grade of CPE	No. and state of normal controls	Remarks
1. 120 CPE-negative HeLa and 30 human amnion cell-cultures in complete medium; 14 days inoculation with Mahoney Str. 10^{-2} to 10^{-9} dilution	Pooled, centrifuged supernatants after thermal shock (storage 4° C, reincubation to 37°C)	2 HeLa Flask-cultures, 5 ml undiluted pool 30 min adsorption and 10 ml complete medium. CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
2. 120 CPE-negative HeLa and 30 human amnion cell-cultures in complete medium; 14 days inoculation with Mahoney Str. 10^{-2} to 10^{-9} dilution	Pool of homogenates (cells stored at −20°C, without culture-fluid)	2 HeLa Flask-cultures, 5 ml undiluted pool 30 min adsorption and 10 ml complete medium. CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
3. 49 CPE-negative HeLa and 10 human amnion cell-cultures, as above	Pool of 10% diluted Urea-lysate of cells (stored at −20°C)	5 ml Lysate in 10–3 dilution: procedure and results as above	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
4. About 120 CPE-negative HeLa cell-cultures, as above	'Total lysates': frozen cells dissolved in Urea-reagent and diluted to 10% with *own* medium + H_2O	Assayed after 7 weeks storage at 4°C, Delayed CPE 4-plus, suddenly on 7th day	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
5. 60 CPE-negative HeLa cell-cultures, as above	'Total lysates' as above	Assayed immediately; CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
6. 30 CPE-*positive* HeLa cell-cultures as above	Supernatant separated; cell residue lysed and diluted till 10% Urea-concentration	Assayed immediately; CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive

plication of the same techniques for demonstration of infectious virus in cell cultures *unsuccessfully* inoculated with poliomyelitis virus is now reported. Characteristic enzyme changes were observed in similar TC suggesting the viral cause of biochemical alterations[2]. The following experiments demonstrate that superficial or intracellular association of highly cytopathogenic virus (Type 1, Mahoney str.) may occur *without massive cell destruction and cytological changes* visible on simple microscopic inspection. About 120 tubes of individual subcultures of HeLa and primary cultures of human amnion cells exposed to virus dilutions from 10^{-2} to 10^{-9} were collected which *did not show any signs of cytopathogenic effect* (CPE), even after 10 to 14 days incubation at 37°C. These TC's were allowed to stand 2 days at 4°C, then 2 days at 37°C. The supernatants were pooled, centrifuged, and assayed separately. The unwashed cells were homogenized by a loosely fitting motor-driven pestle of an all-glass micro-grinder (Potter-Elvhejem type) in about 1 min. runs. The pooled homogenates were assayed for infectivity; two TC's, each with ab. 3×10^6 HeLa cells grown with 15 ml complete medium[3] were inoculated with 5 ml undiluted 'brei'. Two other cultures received 5 ml of the pooled *supernatants* to test the presence of virus in the fluid-phase. Usually 30 min adsorption of the inoculum was allowed at 37°C, then 10 ml fresh medium was added and the TC reincubated at 37°C. Inspection after 18 h revealed definite CPE leading to total destruction of the TC's within 2–3 days. It was concluded that both the pools of *supernatants* and *homogenates* contained highly infectious virus material.

This suggestion was proved by chemical means. 60 CPE-negative TC's of HeLa and primary human amnion cells were frozen without nutrient-fluid at-20°C, then dissolved by UDC-reagent[2] to demonstrate cell-associated virus. 2 ml UDC were added to five tubes at *room temperature.* The cultures lysed promptly and were poured over into other tubes. This procedure was repeated several times back and forth, finally all lysates were pooled, the tubes

[2] E. KOVÁCS, Z. Vitamin-, Hormon-Fermentforsch. *4* (1959).

[3] J. L. MELNICK, Ann. N. Y. Acad. Sci. *61*, 754 (1955).

washed with H_2O, the lysates diluted 10-fold (10% final urea conc.) with the rinsing-water[2] as soon as possible and stored at 4°C. The whole procedure involved about 60 min. For inoculation, a 10^{-3} dilution with bovine amnion fluid was made and assayed as described. Control flasks received similarly diluted UDC. The TC exposed to lysates were destroyed within two days, whereas controls treated with urea-desoxycholate alone remained intact, permitting growth and subcultivation of the cells. It seemed that the lysates were carrying (among others) *biologically active virus material* set free by UDC and preserved by the timely dilution[2]. This assumption was substantiated by the following experiments. *The cell residues of positively infected and destroyed (CPE-positive) TC* were dissolved in UDC as usual and their preserved infectivity demonstrated. HeLa cells inoculated with this lysate exhibited visible CPE after 16 h and the whole culture was destroyed within two days. Finally if 10% Urea-lysates were added to the TC and after 5 min diluted 3 times with amnion fluid, immediate destruction of the cells by the UDC occurred. This was proved with the large 'total' pool illustrated in the Table (group 4) where medium and cells were lysed together. The tenfold diluted lysates, however, do not retain intactly their infectivity during 7 weeks storage at 4°C; if tested in 10^{-3} dilution, CPE was not apparent only after 6 days, when overnight total destruction of the inoculated TC occurred. Second passages of the cultures destroyed were positive, confirming the presence and successful transmission of infective material with homogenates and lysates (Table). Concentration, purification, and isolation of the active principle is under study[4].

We might summarize that the above findings were repeatedly observed and may prove the following assumptions; (1) The CPE-negativity of unsuccessfully infected TC may depend on a peculliar behaviour of the cells against polio virus (2). The infective particle seems to be firmly associated to certain cells without apparent cytopathogenic effect, and can be liberated by *physical* (thermal-shock, grinding) and/or *chemical* means (3). UDC-treatment *did not destroy the biological activity* of the virus examined (4). The UDC reagent seems to be the simplest *chemical* tool[5] for the liberation of infectious material from true or latent poliomyelitis infection *in vitro*, although the nature of this procedure has to be clarified.

The technical help of Mrs. VICTORIA STÜRTZ in one phase of the work is gratefully acknowledged.

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Zusammenfassung

Stark cytolytisch wirkende Urea-Desoxycholatlösung zertört die biologische Aktivität des Poliovirus (Typ I, Mahoney-Stamm) *nicht;* somit ist dieses Mittel zur Freisetzung infektiösen Materials aus latent oder manifest infizierten Zellkulturen sehr geeignet.

[4] In preparation.

[5] J. S. COLTER, *Nucleic Acid as Carrier of Viral Activity* in E. BERGER and J. L. MELNICK, *Progress in Medical Virology*, vol. I (S. Karger, New York–Basel 1958), p. 1.

Metabolic Interactions In Vitro between Polymorphonuclear Leukocytes and Pathogenic and Nonpathogenic Microorganisms[1]

The addition of bacteria to polymorphonuclear leukocytes in the Warburg respirometer causes an alteration of the otherwise constant rate of oxygen uptake by the leukocytes. Changes of rates may be attributed to the effect exerted by bacteria on the leukocytes and by leukocytes on the phagocytized bacteria[2–4]. In previous studies it had been shown that tubercle bacilli maintained a constant rate of respiration in an intracellular environment as evidence of their ability to survive within phagocytic cells[5]. These investigations were extended to other pathogenic and nonpathogenic bacteria. The results of these studies are reported.

Methods. Bacteria were grown in brain-heart-infusion broth and were washed repeatedly. Peritoneal exudate leukocytes from rabbits were obtained by injecting a solution of sodium caseinate intraperitoneally 15 h prior to collection. The cells were washed twice in saline; leukocytes and bacteria were resuspended in a medium consisting of Krebs Ringer phosphate buffer containing 20% homologous, heat-inactivated serum, and 100 mg% glucose. The Warburg vessels were prepared as follows: Leukocyte suspension in main chamber, bacterial suspension (live or heat-killed bacteria) in side-arm, and 10% NaOH in center well. After equilibration in the water bath, the bacteria were added to the leukocytes by tipping the content of the side-arm into the main chamber. Manometric readings were taken every 30 min over a period of 3 to 4 h. In every experiment leukocytes alone, bacteria alone, and leukocytes with live and dead bacteria were used. Samples of mixtures and bacterial suspensions were removed from the flasks to determine number of viable bacteria and extent of phagocytosis.

Results. When oxygen uptake of leukocytes alone with that of leukocytes with *heat killed* bacteria was compared, the results represented in Table I were obtained. The average number of leukocytes per flask was 1×10^8, and there were about 2 to 5 times as many heat killed bacteria. By dividing the rate of oxygen consumption of phagocytes with bacteria by the rate of consumption by phagocytes alone, a ratio is obtained which indicates the difference between the two sets of flasks. As can be seen, the leukocytes showed increased respiration (up to 50%) after having ingested heat killed, *nonpathogenic* bacteria, whereas a reduction of oxygen consumption occurred when the leukocytes had phagocytized heat killed, *pathogenic* organisms.

The respiration of living intracellular bacteria was calculated indirectly, assuming identical oxygen consumption by phagocytes regardless of whether or not the ingested organisms were living or dead. Including the necessary control flasks, one could obtain values for oxygen consumption by bacteria within phagocytes and by bacteria suspended in cell free media. The ratios of these two

[1] This investigation was supported by Grant E-1302 (C) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

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[3] H. STÄHELIN, E. SUTER, and M. L. KARNOVSKY, J. exp. Med. *104*, 121 (1956).

[4] E. H. PERKINS, F. MIYA, and S. MARCUS, Fed. Proc. *17*, 529 (1958).

[5] H. STÄHELIN, M. L. KARNOVSKY, and E. SUTER, J. exp. Med. *104*, 137 (1956).