until a constant melting point was attained. The colorless platelets melted at 77°C and were soluble in chloroform, ethyl ether, water, and methanol. Sodium fusion indicated the absence of halogens, sulfur, and nitrogen. A molecular weight of 268 was obtained by the Rast camphor method, and elemental analysis showed that the compound contained 63.94 percent carbon and 5.86 percent hydrogen. These data suggest an empirical formula of $C_{15}H_{15}O_5$. The ultraviolet absorption spectrum in methanol revealed a maximum at 268 mμ and minima at 242 mμ and 287 mμ. The infrared spectral analysis in chloroform showed a maximum at 6.01 μ.

A search of the literature failed to reveal a discussion of a compound approximating $C_{15}H_{15}O_5$ and having the previously mentioned properties.

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23 July 1956

New Chemical Method to Differentiate Human-Type Tubercle Bacilli from Other Mycobacteria

No morphological or immunological method to differentiate the various types of mycobacteria exists at the present time. All attempts to find a type-specific skin reaction have also failed. The only methods in current use are based on cultural and pathogenic characteristics. This paper describes chemical methods that are dependent on the metabolic properties of mycobacteria.

Pope and Smith (*1*) determined the vitamin-B production in the culture filtrate of human and bovine tubercle bacilli, respectively, grown on synthetic liquid media. Bird (*2*) also measured the vitamin-B content of human tubercle bacilli.

Konno and coworkers (*3*) reported on a marked quantitative difference in the niacin production by human-type tubercle bacilli and other mycobacteria when grown on synthetic culture media. This difference is strictly linked to the type of the bacillus.

The present investigation aims at simplification of the previous method by using colonies of bacilli taken directly from the routine solid diagnostic media. The following materials were used: 15 strains of standard laboratory mycobacteria (Table 1), 50 strains of tubercle bacilli obtained from tuberculous patients, including three isoniazid-resistant, catalase-deficient strains, which did not cause progressive tuberculosis in guinea pigs, and 10 strains of "atypical acid-fast bacilli," most of them chromogens (Table 2). These laboratory and clinical materials were inoculated on the Loewenstein-Jensen's solid culture media and allowed to grow from about 1 to 3 months.

For the estimation of niacin, the aniline and cyanogen bromide method (*4*) is used; a few colonies are carefully taken from the culture medium by a platinum loop and transferred into a test tube that contains 1 ml of 4-percent aniline solution (*5*). To this is added 1 ml of 10-percent cyanogen bromide (*6*). A positive test shows the development of an intensive canary-yellow color first noticed in the bacterial sediment of the test tube and later, after shaking, in the supernatant fluid. A positive test is developed only by human-type tubercle bacilli, whereas the other mycobacteria (bovine, avian, nonpathogenic mycobacteria and the group of "atypical acid-fast bacilli") do not develop an appreciable yellow color.

Controls with malachite green-containing medium showed a green discoloration of the fluid. Controls with aniline solution and bacilli or cyanogen bromide solution and bacilli show no color production. The color production by this aniline-cyanogen bromide method is more intense than the previously used metol and cyanogen bromide method (*7*) or the ammonia buffer and cyanogen bromide method (*8*).

As mentioned before only human-type tubercle bacilli give a positive test, irrespective of virulence. This was confirmed with standard laboratory strains as well as with strains obtained directly from patients; no difference in the test was noted between isoniazid-sensitive or resistant, catalase-producing or deficient strains. Bovine tubercle bacilli, whether highly virulent or as attenuated as the B.C.G., show a negative test. Avian tubercle bacilli, nonpathogenic bacteria, and all "atypical acid-fast bacilli" studied to date give negative tests.

Table 1. Niacin test of standard laboratory mycobacteria.

Type	Strain	Niacin test
Human tubercle bacilli	Virulent	
	1) H37 Rv	+
	2) Campbell	+
	3) Erdman	+
	Attenuated	
	4) H37 Ra	+
	5) JH 16 Ra	+
	6) JH 6 Ra	+
Bovine tubercle bacilli	Virulent	
	7) Vallée	−
	8) Ravenel	−
	Attenuated	
	9) B.C.G.	−
Avian tubercle bacilli	10) Sheard	−
	11) Camden	−
	12) Avian I	−
Nonpathogenic mycobacteria	13) *M. ranae*	−
	14) *M. phlei*	−
	15) M. 607	−

Table 2. Niacin test of tubercle bacilli and "atypical acid-fast bacilli" obtained from patients.

Fifty strains of tubercle bacilli including three isoniazid-resistant, catalase-deficient strains	Niacin test positive 50	Niacin test negative 0
Ten strains of "atypical acid-fast bacilli"	Niacin test positive 0	Niacin test negative 10

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5. Four-percent aniline in 96-percent ethylalcohol is practically colorless.
6. Dissolve 50 g of cyanogen bromide in 500 ml of distilled water. This reagent is colorless. Both reagents remain stable for several months if they are kept in a brown bottle and in the refrigerator.
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22 August 1956